CEREAL CHEMISTRY

VOL. XVIII MARCH, 1941 No. 2

PHYSICAL ASPECTS OF STARCH BEHAVIOR

THOMAS JOHN SCHOCH

Corn Products Refining Company, Edgewater, New Jersey

(Read at the Annual Meeting, May 1940)

Until recently the various colloidal characteristics of a starch paste were attributed to the influence of specific component substances which presumably retained their identity under widely varying conditions. Due in large part to the association theory formulated by Meyer and Mark (1930), this has been supplanted by the concept of starch as one of the most complex of colloids, whose behavior is affected as much by physical environment as by actual chemical reactions.

With large molecules heavily loaded with hydrophilic groups, there arise associative forces which may approach or even exceed the stability of primary chemical linkage. The characteristic birefringence of the ungelatinized starch granule under polarized light is sufficient indication that the molecules have been deposited in some sort of orderly arrangement. Under such circumstances, the associative forces of adjacent molecules would be mutually satisfied, hydration tendencies would be at a minimum, and consequently the granule would be insoluble in cold water. If a water suspension of starch is slowly heated, the granules lose their birefringence before swelling becomes pronounced. This could be ascribed to partial hydration of the starch molecules, thereby disrupting their orderly arrangement. As heating is continued, the granules swell until they can no longer be readily distinguished under the microscope. Only then does the viscosity commence to rise. These changes are readily followed with the Brabender amylograph, whereby viscosity is charted against uniformly rising temperature. Figure 1 shows such a curve for raw corn starch, replotted on ordinary cross section paper. Samples were withdrawn at regular intervals and examined under the polarizing microscope.

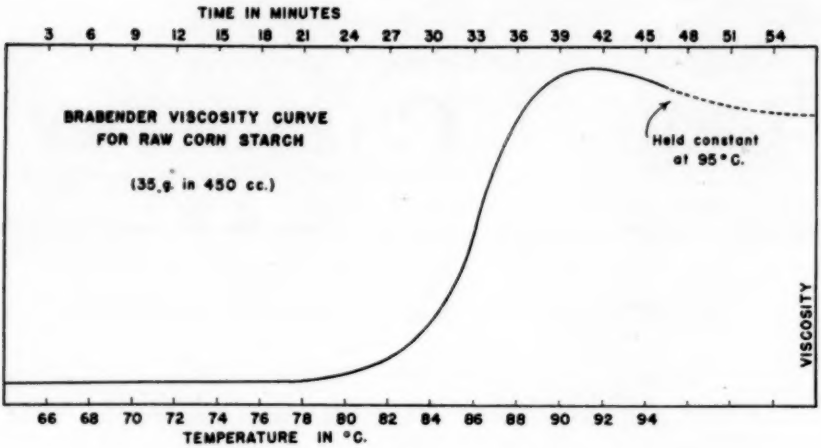
TIME IN MINUTES
3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54
BRABENDER VISCOSITY CURVE
FOR RAW CORN STARCH
(35 g. in 450 cc.)
Held constant
at 95°C.
VISCOSITY
66 68 70 72 74 76 78 80 82 84 86 88 90 92 94
TEMPERATURE IN °C.

Figure 1.

Temperature	*Microscopic Appearance*
64° C.	No apparent effect.
66° C.	Slight swelling. Approximately 25% of granules have lost birefringence.
68° C.	Approximately 75% of granules have lost birefringence.
70° C.	Very few birefringent granules. Swollen approximately 2 ×.
72° C.	No birefringent granules.
74° C.	Swollen approximately 3 ×.
76° C.	Same.
78° C.	Slight additional swelling.
80° C.	Swollen approximately 4 ×. Granule outlines becoming vague.
82° C.	Same.
84° C.	Very vague.

However, much of the viscosity of a boiled starch paste must be attributed to swollen aggregates or to invisible fragments of granule structure, since such pastes can be radically thinned by autoclaving or by violent mechanical agitation. On prolonged autoclaving, the viscosity approaches a minimum which represents maximum dispersion of the starch. Such thinned pastes cannot be considered as true solutions, since the associative forces are still potentially active.

Water is not the only medium in which starch can be gelatinized. Liquid ammonia, liquid HCN, formamide, and formic acid are reputed to be excellent gelatinizing media. It will be noted that these are all liquids of high dielectric constant—in other words capable of dissociating the hydrophilic cohesion between starch molecules. Dissociation may likewise be effected by substances which are preferentially adsorbed on the hydroxyl groups of the starch, thus neutralizing intermolecular attraction. Sodium hydroxide solutions, above 1% concentration, will

gelatinize starch in the cold to give thick pastes or rubbery gels. This is merely a matter of adsorption, since the alkali can be completely removed by repeated precipitation with methanol. Saturated sodium hydroxide, on the contrary, will not gelatinize starch, possibly because it does not afford sufficient free water for hydration. Gels can also be obtained in solutions of certain salts, the familiar Hofmeister series, of which thiocyanates have the highest peptizing action. As in the case of sodium hydroxide, this gelation proceeds only as far as swelling, without any real disintegration of internal granule structure to produce a solution. The sodium hydroxide gel can be liquefied by heating, but this obviously involves chemical decomposition. However, if the peptizing ion carries a large inert residue, as in the case of the quaternary bases, then the solution state is more closely approached. For example, while starch is not affected by cold ammonium hydroxide, it readily dissolves in a cold aqueous solution of trimethyl benzyl ammonium hydroxide to give a limpid solution of low viscosity. Lieser and Ebert (1937) have observed a similar effect with cellulose, and they find that the peptizing activity rises with increasing molecular size of the quaternary base. They suggest that the quaternary ammonium ion acts as a wedge to pry apart the individual carbohydrate molecules. Morpholine, trimethylamine, and ethylene diamine have a similar action in hot solution, in these instances without apparent alkaline decomposition. Here it may be the hydrophilic amine group which associates with the starch by a process akin to hydration.

The solubility of starch can be raised by certain chemical modifications which might be mentioned at this point, though the mechanism of the reactions involved cannot as yet be satisfactorily explained. For example, when starch is dextrinized by heat, its solubility is markedly increased. There is some reason to believe that molecular water is eliminated to give an internal glucosan structure, and such products might be expected to show decreased associative tendencies. Even more obscure is the action of oxidizing agents. Treatment of raw starch with alkaline hypochlorite or peroxide increases solubility and decreases viscosity. Some oxidation of primary alcohol groups might be expected—in fact traces of uronic acids have been detected on hydrolysis of these starches—but this is scarcely sufficient in itself to explain the considerable change in character. With thin boiling starches produced by acid treatment, the manifest reaction is one of glucosidic hydrolysis, reducing the size of the starch molecule down from its highly associative colloidal dimensions. However, there is evidence of some type of linkage in starch which is even more susceptible toward acid hydrolysis than is the normal glucosidic bond. The solubility of certain starch ethers can be

best explained by the association theory. If an alkaline starch paste is treated with propylene oxide, the resulting hydroxyalkyl derivative yields clear aqueous solutions of high stability. Since the free hydroxyl content has not been affected, and since there is no possibility of glucosidic hydrolysis, the enhanced solubility can only be explained as a sort of steric hindrance, the substituent groups interfering with association of the starch molecules. The effect is even more pronounced with ethylated starch, where the free hydroxyl content has been reduced.

When granule structure is disintegrated by prolonged dry-grinding in a ball mill (Alsberg and Perry, 1924), the viscosity is markedly reduced. Unfortunately, the so-called alkali lability increases (Taylor, Fletcher, and Adams, 1935), indicative of some sort of hydrolytic scission. In this connection, it might be remarked that the efficiency of granule disintegration is greatly improved by wet-grinding an alcohol slurry of the starch. But even by this method, the alkali lability rises somewhat.

In a certain sense, the selective action of certain solvents in removing fatty material from the cereal starches is indicative of the hydrophilic bonding within the granule (Schoch, 1938). Only those fat solvents of a hydrophilic character can penetrate into the granule. If bone-dry starch is confined over various volatile liquids, vapor adsorption is dependent on the hydrophilic nature of the liquid. In discussing the extraction of chlorophyll from green leaf tissue, Bancroft and Rutzler (1938) suggest that only those solvents which are preferentially adsorbed by the cellulosic leaf tissue can desorb the chlorophyll, with consequent removal of the latter. A similar mechanism should apply to the fatty acids and phospholipids in the various cereal starches.

If starch is first peptized by alkali, or by the quaternary bases, or by salts of the Hofmeister series, then the peptizing agent removed (as by alcohol precipitation, or electrodialysis, or neutralization with acid), the starch is thrown out as a mushy paste. With trimethylamine, a similar phenomenon results if the volatile base is distilled off. All such tendencies of starch to revert to less soluble forms are given the broad designation of "retrogradation," paralleling the denaturation of proteins. Examples are plentiful. If an autoclaved starch paste—representing the maximum degree of dispersion which can be achieved without hydrolytic degradation—is allowed to stand at room temperature, there is a gradual flocculation of insoluble material. To a limited extent, this can be re-dispersed by further autoclaving. Retrogradation proceeds more rapidly, and is more complete at low temperatures. If a starch paste is frozen, then thawed, it gives a spongy fibrous mass representing the total original starch (Woodruff and MacMasters, 1938). This may be

partially dissolved by autoclaving, but each subsequent freezing renders it more resistant toward solution. If we conceive of freezing as dehydration by ice formation, then association between starch molecules could reassert itself.

The speed of retrogradation is dependent on the concentration of the starch paste or solution. Thus an autoclaved 1% starch solution will remain fairly clear for several days, while a 2% solution will flocculate over night. Solutions of 3% are highly unstable, and may gel out spontaneously in an Ostwald pipette during a viscosity determination. If an autoclaved 1% solution is evaporated *in vacuo* on the steam bath, it will suddenly revert to an insoluble mush when the concentration reaches 3% — 5%. Addition of water at this point does not effect re-solution.

Another instance of retrogradation is the skinning of boiled starch pastes through surface evaporation. The significant point is that such skins are very difficult, if not impossible, to redisperse.

Alcohol precipitation of starch from pastes or solutions may be considered as involving retrogradation. In the first place, such precipitation is in marked contrast to that of crystalloids where there is a definite solubility at each concentration of alcohol and water. If methanol is slowly added to an autoclaved starch solution, there is no pronounced change until the alcohol concentration reaches approximately 40%, when the solution becomes opaque. When the concentration reaches 45%, the starch is completely flocculated. Attempts to separate possible starch components by such fractional precipitation have not been successful, because of this very narrow range. The concentration of various precipitants necessary to initiate flocculation is significantly in line with the dielectric constant:

Acetone or diacetone	32%
Methyl or ethyl alcohol	38%–40%
Methyl cellosolve or dioxan	53%–56%
Ethylene glycol or glycerol	above 70%

Starch which has been precipitated by alcohol cannot be redissolved to give anything approaching its former degree of dispersion, even by autoclaving. Such retrograded starch can be dissolved in alkali, though with some difficulty. This might be anticipated, since alkali-peptized starch does not retrograde on freezing or on standing, nor does it exhibit the skinning effect observed with starch pastes.

Retrogradation is favored by low pH. Solutions of starch which have been buffered to various pH levels and allowed to stand will show a progressive increase in precipitation as the pH is lowered. Below a pH of 3, the amount of precipitated material diminishes, due to hydrolysis of the starch.

Certain polar substances containing a heavy hydrophobic loading cause precipitation of starch, though in somewhat different fashion. Cyclohexanol is particularly effective in this respect, precipitating the starch completely and immediately from hot solution. This differs from normal retrogradation in that the starch redissolves when the cyclohexanol is removed by steam distillation. This action might be visualized as adsorption of the cyclohexanol through association of its hydroxyl group with those of the starch, thus coating the latter with a waterproof film of oriented cyclohexane radicals and preventing hydration. A number of wetting agents have a similar action, as well as fatty acids and higher alcohols, soaps, sulfonated oils, etc. Familiar to the starch chemist is the fact that a tapioca starch paste—normally slimy and transparent in character—is converted into a " short " opaque paste by the addition of a small amount of sulfonated castor oil. The slimy nature of tapioca starch can only be attributed to a high degree of molecular association, approaching that of a gel. If a dilute tapioca paste is rapidly stirred to impart a swirling motion, this is quickly damped, then a reverse motion is set up, and the paste may wind and unwind for several seconds. This would indicate a skeleton of molecular forces operating throughout the paste. Now if a sulfonated oil is added, the paste loses most of this back-lash effect, behaving more as if it were composed of discrete particles whose only relation to one another was one of mechanical friction. Some of the physical differences between the tuber and cereal starches can be ascribed to the presence of polar fatty acids in the latter. Cornstarch pastes are shorter, less slimy, and more opaque than those from tapioca starch. However, if the fatty acids are removed from the cereal starches by Soxhlet extraction with methanol, then the resulting starch yields pastes which are much more transparent and slimy. As another example, a 5% paste of raw rice starch must stand for a day or more to give a weak gel, while a similar paste of de-fatted rice starch sets up to a strong gel immediately on cooling to room temperature.

The action of borax to produce rubbery gels with solutions of starch or vegetable gums is well known. Electrometric titration curves indicate that the ionization of boric acid is increased by the addition of starch or dextrin. So the gelling action might well be related to the complex formation between borate ion and such polyhydroxylated substances as glycerol, glucose, and mannitol. If borate acted as a bridge between adjacent starch molecules, the anticipated effect would be an increase in viscosity and gelling characteristics.

Those modified starches whose solubility has been enhanced by such means as dextrinization, or oxidation, or ethylation, show little tendency

to retrograde. Solutions are stable over extended periods of time, do not readily skin over, or if a skin does form it can be readily dissolved. They are less sensitive toward precipitation by polar substances, and when flocculated by excess alcohol, can be readily redissolved in water.

All this adds up to a fairly reasonable and consistent picture. The affinity of starch for water is opposed by the more powerful association forces operating between the starch molecules. Consequently, hydration proceeds only far enough to give colloidal gels, or with extreme difficulty to give colloidal solutions. These are meta-stable, and require little provocation to revert to the more stable insoluble state. More complete dissociation of the starch molecules can only be accomplished by agents possessing greater ability than water to satisfy the intermolecular forces. The various degrees of starch dispersion may be crudely visualized as a sort of spectrum:

MONOMOLECULAR DISPERSION

↑Heat	Dextrins and modified starches
↓Time	Solutions in quaternary bases
↓Concentration	Autoclaved pastes
↓Acidity	Sodium hydroxide gels
	Thiocyanate gels
	Boiled pastes
	Raw starch
	Frozen starch
	Alcohol-precipitated starch
	Starch "skins"

COMPLETE RETROGRADATION

Few investigators have ever agreed on the ratio of soluble and insoluble amyloses in starch, estimates running the entire gamut from 100% amylose to 100% amylopectin, depending on the mode of isolation. With such a meta-stable substance as starch, it is obvious that the amount of any such fraction would be governed by the degree of dispergation of the granule, the amount of retrogradation which occurred, the effect of chemical degradation (as by acid hydrolysis), and a host of similar factors to which little heed has been given. As a result of this confusion, there has lately been a trend to disregard such possible components and to consider starch as a single chemical entity.

But recent evidence may reopen this question, since fractions have been isolated from starch which are markedly different, not only in physical characteristics but likewise in chemical reactivity. When an autoclaved starch solution is treated with excess butyl or amyl alcohol, there occurs a slow precipitation at the alcohol-water interface. This material is semi-crystalline in appearance, minute spheroids or needles,

quite different from the amorphous floc of retrograded or methanol-precipitated starch. The supercentrifuge is the only satisfactory means of isolating this product, to give a creamy white deposit. If this is dehydrated with alcohol, it becomes completely insoluble. However, if removed from the centrifuge and immediately treated with boiling water, it readily dissolves to a clear thin syrup, even at concentrations as high as 20% solids. Such a solution shows greatly exaggerated retrogradation tendencies. If allowed to cool even slightly, it sets to a rubbery translucent gel which cannot again be liquefied by heating. It can be purified by reprecipitation with butanol, consistently amounting to 21%–23% of the original starch. The remainder of the starch can be precipitated from the centrifuged solution with methanol. In contrast, this material is readily soluble in water to give clear stable syrups. But the most significant difference lies in their respective alkali labilities, the insoluble fraction being much more labile than is the parent raw starch, while the soluble fraction is correspondingly more alkali-stable. Such behavior can only be ascribed to variation in chemical configuration. This study is being continued, in an effort to identify the structural differences.

Literature Cited

Alsberg, C. L., and Perry, E. E.
1924 The effect of grinding on starch and starch pastes. Proc. Soc. Exp. Biol. Med. **22**: 60–61.

Bancroft, W. D., and Rutzler, J. E., Jr.
1938 The colloid chemistry of leaf and flower pigments. I. The precursors of the anthocyanins. J. Am. Chem. Soc. **60**: 2738–2747.

Lieser, Th., and Ebert, R.
1937 Cellulose and its solutions. Ann. **528**: 276–295.

Meyer, K. H., and Mark, H.
1930 Aufbau der hochpolymeren organischen Naturstoffe. Akad. Verlagsges. Leipzig.

Schoch, T. J.
1938 Absence of combined fatty acid in cereal starches. J. Am. Chem. Soc. **60**: 2824–2825.

Taylor, T. C., Fletcher, H. H., and Adams, M. H.
1935 Determination of the alkali-labile value of starches and starch products. Ind. Eng. Chem. (Anal. Ed.) **7**: 321–324.

Woodruff, S., and MacMasters, M. M.
1938 Gelatinization and retrogradation changes in corn and wheat starches shown by photomicrographs. Ill. Agrl. Exp. Sta. Bull. 445, 43 pp.

A METHOD FOR THE DETERMINATION OF REDUCING MATTER IN FLOUR

J. FREILICH

The Fleischman Laboratories, Standard Brands Incorporated, New York, N. Y.

(Read at the Annual Meeting, May 1940)

In connection with our work on the effects of oxidation in doughs, it is of great interest to know the relative quantities of reducing matter present in different flours, since it is known that such substances stimulate protease activity, and we have shown that additions of reducing factors such as those present in wheat germ produce great differences in baking quality.

An examination of some of the commonly used books on food analysis revealed no detailed method for the determination of reducing matter in products such as flour and cereals. Brief outlines of two such methods were found in a paper by Sullivan, Howe, and Schmalz (1937). By one method, the proteins are removed with sodium tungstate and sulfuric acid; then a titration is made with KIO_3 in KI and thiosulfate, using a potentiometer. By the other method, an iodine titration is used after removal of the proteins by use of sulfosalicylic acid.

Woodward and Fry (1932) found that autoxidation with loss of reducing matter took place in tungstic acid filtrates even at pH 3.0, and that with trichloracetic acid the recovery of added glutathione was incomplete, but that with sulfosalicylic acid there were both good recovery and no autoxidation.

Preparation of Concentrated Flour Extracts

The availability of the Waring Blendor, an efficient, high-speed stirrer, made it possible to use flour extracts more concentrated than those used by Sullivan in the tungstic acid method.

Table I shows the variations in iodine uptake between extracts prepared as in the tungstate procedure and more concentrated extracts, prepared with the aid of the Waring Blendor.

These results showed: (1) that the use of the Waring was as good as hand shaking in the tungstate procedure, (2) that the Waring gave considerably more reducing matter in the more concentrated extract as compared to hand shaking, and (3) that the value for reducing matter in the more concentrated extract was very much greater than in the Sullivan procedure.

TABLE I

AMOUNTS OF IODINE SOLUTION TAKEN UP BY FLOUR EXTRACTS OF DIFFERENT CONCENTRATIONS, PREPARED BY MANUAL SHAKING COMPARED TO MECHANICAL STIRRING AND CENTRIFUGING

	Iodine solution used by 100 cc. of flour extract (cc. of 0.010*N*) [1]
One part flour, 5 parts water:	
Shaken by hand (tungstate procedure)	1.05
Stirred in Waring for 1 minute	1.1
One part flour, 3 parts water:	
Shaken by hand, centrifuged	1.5
Stirred in Waring, centrifuged	1.9

[1] The titrations were made by adding 10.0 cc. of the iodine solution and titrating back with thiosulfate solution, using starch indicator.

Effect of pH on the Amount of Iodine Taken Up by Flour Extracts

That the control of pH is of prime importance was indicated by tests in which a plain water extract of a low-grade flour took up six times as much iodine as an equal quantity of the same extract in which tungstic acid was used.

The effect of pH on the amounts of iodine taken up by extracts of patent and low-grade flours are shown in Table II and Figure 1. The flour extracts were prepared by stirring 1:3 flour-in-water suspensions in the Waring Blendor, centrifuging, boiling the liquids, and filtering off the coagulated protein; the filtrates were made up to volume, and 50-cc. portions were titrated after additions of acid to give increasingly lower pH values.

It is evident that there is a marked decrease in the quantity of iodine taken up by the reducing matter in the extracts with decreasing pH, and

TABLE II

EFFECT OF pH ON AMOUNT OF IODINE TAKEN UP BY EXTRACTS OF PATENT AND LOW-GRADE FLOURS

Patent		Low grade	
pH	Iodine used	pH	Iodine used
	cc.		*cc.*
5.92	2.1	6.1	4.4
4.72	1.2	5.42	3.2
4.30	0.9	4.9	2.6
3.87	0.8	4.2	1.7
3.55	0.6	3.18	1.2
3.0	0.6	2.65	1.1
2.7	0.6	2.37	1.05
2.3	0.6	1.9	0.9
1.9	0.55		

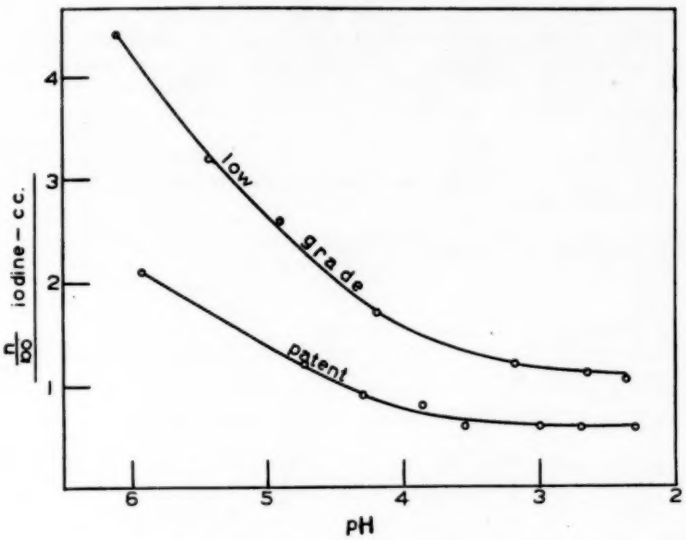

Fig. 1. Effects of increasing acidity on the amount of iodine taken up by flour extract.

that the values for each flour tend to become constant below pH 3.0. It is therefore evident that for comparable results, titration should be made in highly acidified extracts.

Because of the high buffer value of flour, and the great differences in buffer value between patent and low-grade flour, it was indicated that the most desirable procedure was probably the addition of a large enough excess of acid to give a sufficiently low pH even with the most highly buffered flours.

Factors in Loss of Reducing Matter through Oxidation

That appreciable amounts of reducing matter were lost, probably through oxidation, was indicated by experiments in which (1) variations were made in time of stirring in the presence of air, (2) air in the flour was replaced by nitrogen before stirring, and (3) titrations were made at different times after starting the filtration of the liquid following centrifuging. The results of these experiments are shown in Table III.

The figures in Table III indicate (1) a slight increase in reducing matter due to removal of air from the flour, (2) a great loss in reducing matter due to long stirring while the suspension is exposed to air, and (3) slight loss in reducing matter due to standing exposed to air during and after filtration.

To avoid exposure to air during the stirring of the suspension, the edge of the Waring container was ground smooth so that a piece of flat

TABLE III

EFFECTS OF AIR IN THE FLOUR, THE TIME OF STIRRING OF THE SUSPENSION, AND THE TIME OF TITRATION AFTER THE START OF FILTRATION, ON THE AMOUNTS OF IODINE TAKEN UP BY FLOUR EXTRACTS

	Iodine used (cc. of 0.010*N*)
Air in the flour:	
Flour stirred in an atmosphere of nitrogen to remove air	1.85
Flour not treated	1.70
Time of stirring in Waring:	
1 minute	3.2
5 minutes	1.9
Time of titration after start of filtration:	
15 minutes	3.50
75 minutes	3.35
135 minutes	3.25

plate glass would fit tightly over it; it was then completely filled with the suspension so as to exclude as much air as possible.[1]

Time of Standing after Stirring

Tests in which the suspension was allowed to stand for varying time intervals before centrifuging, gave results as shown in Table IV.

TABLE IV

AMOUNT OF IODINE TAKEN UP BY FLOUR EXTRACTS AS AFFECTED BY THE TIME OF STANDING AFTER STIRRING AND BEFORE CENTRIFUGING THE SUSPENSION

Time	Iodine used (cc. of 0.010*N*)
None	2.7
½ hr.	2.9
1 hr.	3.3
2 hrs.	3.3

The results showed that the suspension should stand about one hour before being centrifuged, to obtain close to maximum extraction.

Time between the Addition of the Iodine Solution and the Back Titration with Thiosulfate

It was found that the longer the extracts stood after the addition of iodine and before the back titration with thiosulfate, the greater was the amount of iodine taken up by the extract. Typical examples of this are shown in Table V.

[1] Thanks are due to Dr. Quick Landis for this suggestion and for performing a difficult piece of work in cutting and grinding the glass container.

TABLE V

EFFECT OF TIME ON THE AMOUNT OF IODINE TAKEN UP BY FLOUR EXTRACTS AND SOLUTIONS OF GLUTATHIONE

	Iodine used (cc. of 0.010*N*)
Flour extracts:	
Titrated immediately	2.2
Titrated after standing 2 hrs. with added iodine solution	3.5
Solutions of glutathione:	
Glutathione, 10 mg.—titrated immediately	3.25
Glutathione, 10 mg.—titrated after standing 1½ hrs. with added iodine solution	3.9

It is therefore evident that the back titration with thiosulfate should be made as soon as possible after the addition of the iodine in order to avoid error. That the value after immediate titration is correct is apparent from the glutathione titration; multiplying 3.25×3.07—which is the mg. equivalent to 1 cc. of 0.010*N* iodine solution—the result, 9.98, is equal to the 10 mg. of glutathione actually used in the titration.

Use of Iodine Solution Compared to Potassium Iodate

In making titrations with standard KIO_3 and an excess of KI, the values obtained were found to be much lower as compared to iodine solution of the same normality. This was very puzzling at first; it was later found, however, that the iodate readings varied in part as the amount of KI used. After equalizing the amounts of KI used, the values for iodate and iodine solution were found to agree.

Table VI shows the differences between iodine solution and KIO_3 of the same normality, as affected by the amounts of KI used with the iodate. Figure 2 shows the effects of varying amounts of KI on the amount of iodine taken up by the wheat-germ extract.

TABLE VI

EFFECTS OF VARYING AMOUNTS OF KI USED IN TITRATIONS OF WHEAT-GERM EXTRACTS WITH IODINE SOLUTION AND KIO_3 OF THE SAME NORMALITY

Standard solution:	Amount of KI in 10 cc. of the solution:	Iodine used by wheat-germ extract equivalent to 1.67 g. germ (cc. of 0.010*N*)
Iodine, 0.010*N*	0.24 g.	2.15
KIO_3, 0.010*N*	Large excess	1.3
KIO_3, 0.010*N*	0.25 g.	2.1
Increasing amounts of KI with 10 cc. of 0.010*N* KIO_3:		
KI (g.)		
0.2		2.15
1.0		1.8
2.0		1.6
4.0		1.45

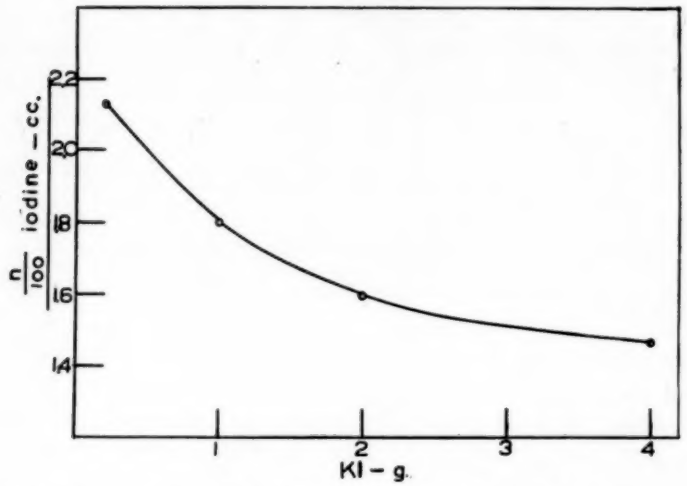

Fig. 2. Effect of increasing amounts of KI on the amount of iodine taken up by wheat-germ extract.

It is evident from these results that the use of large excesses of KI must be avoided in titrations with flour extracts. (The use of large excesses of KI made no difference in titrations with sodium thiosulfate.)

Use of Sulfosalicylic Acid

After finding that 6 g. of sulfosalicylic acid per 100 g. of flour would produce satisfactory precipitation of the proteins, it was compared to tungstic acid, and to boiling and filtration, to note which was best suited for the purpose of protein removal. It was found that boiling and filtration did not remove all the proteins, as indicated by further precipitation after addition of sulfosalicylic acid to such filtrates.

It was also found that the rate of filtration was better for sulfosalicylic acid than for tungstic acid, and the former also seemed to give a slightly better end point in the titration.

Because of these advantages, as well as the reduced autoxidation found by Woodward and Fry (1932), it was decided to use sulfosalicylic acid in preference to the tungstic acid procedure.

Effects of NaCl

Efforts to obtain a clearer extract by using NaCl in conjunction with sulfosalicylic acid were not very successful, till it was observed that clear extracts were obtained from doughs containing about 2% NaCl. It was then found that if the NaCl is stirred into the suspension with

the flour, and the sulfosalicylic acid is added after allowing the suspension to stand for some time, fairly clear extracts are obtained. (In the earlier experiments, the NaCl and sulfosalicylic had been added at the same time.)

Method for Reducing Matter in Flour

The method for reducing matter in flour (or dough) as finally adopted, was as follows:

108 g. flour, 2 g. NaCl, and 314 cc. water (these quantities were found to be just enough to exactly fill a small Waring container) are stirred for 60 seconds in the Waring Blendor, after the flour is first stirred dry in an atmosphere of nitrogen. The suspension is allowed to stand in stoppered containers for 45 minutes at 30°C. and 10 cc. of a 60% solution of sulfosalicylic acid is then added to the suspension, which—after slight shaking by hand and standing at 30°C. for another 15 minutes—is centrifuged in stoppered glass centrifuge bottles. The centrifuged liquid is filtered through folded paper, and 100 cc. of the filtrate titrated back with 0.010*N* thiosulfate immediately after adding 10.0 cc. of 0.010*N* iodine solution. Two cc. of 1% starch solution is used as indicator.

Recovery of Added Glutathione

That this procedure probably gives fairly accurate values for reducing matter in flour was indicated by tests in which glutathione was added to flour suspensions. The results of one test are shown in Table VII.

TABLE VII

TITRATION OF GLUTATHIONE ADDED TO FLOUR SUSPENSION

20 mg. glutathione added

	Iodine used by 100 cc. of flour extract (cc. of 0.010*N*)
Flour extract, control	1.95
Flour extract from suspension with added glutathione	3.70

Calculation:

Observed difference due to added glutathione, 3.70 − 1.95 = 1.75.

20 mg. glutathione in water solution took up 6.45 cc. of 0.010*N* iodine.

Calculated value $\frac{100}{338} \times 6.45 = 1.92$.

(338 cc. is total volume of liquid in the suspension, including flour moisture.)

$\frac{\text{Observed diff.}}{\text{Calculated diff.}} = \frac{1.75}{1.92} = 91.5\%$ of added glutathione present in the flour extract.

The presence of more than 90% of the added glutathione, as shown by the titration, indicates a fair degree of accuracy, considering the difficulties involved in the procedure.

Reducing Matter in Flours from Northwestern and Texas Wheat

Using the sulfosalicylic acid and NaCl method as described, the relative values for reducing matter in different grades of flour were found to be those listed in Table VIII.

TABLE VIII

REDUCING MATTER IN DIFFERENT FLOURS

Flours from Northwestern wheat	Iodine used (cc.—0.010*N*)	Flours from Texas wheat	Iodine used (cc.—0.010*N*)
Patent	0.9	Patent (80%)	0.9
Straight	1.2	100%	1.1
Clear	1.5	Clear (15%)	1.4
Low grade	3.5	Low grade (5%)	1.9

The values in Table VIII indicate differences in the amounts of reducing matter in different kinds and grades of flour; the lower grades contained the greater amounts of reducing matter, and the flours from the Northwestern wheat showed more reducing matter than the Texas flours; the Northwestern low grade contained by far the greatest amount of reducing matter.

Summary

A study was made of some factors involved in the measurement of reducing matter in flour by use of an iodine titration method.

Concentrated flour extracts were prepared by stirring 1:3 flour-in-water suspensions in the Waring Blendor (an efficient high-speed stirrer) and centrifuging the suspensions.

It was found necessary to use highly acidified flour extracts in order to obtain constant values for the amounts of iodine taken up by the extracts.

Oxidation, due to contact of the flour suspensions or extracts with air, was found to cause appreciable loss of reducing matter.

A method based on the use of sulfosalicylic acid and NaCl for protein removal, the use of the Waring Blendor, and the exclusion of air, is described.

The method was found to be fairly accurate, as indicated by the titratable values in terms of glutathione added to the flour suspensions.

Significant differences in the amounts of reducing matter in different flours were found by use of this method.

Acknowledgment

The author wishes to acknowledge the advice and assistance given him by Dr. C. N. Frey, director of the Fleishmann Laboratories.

Literature Cited

Sullivan, B., Howe, M., and Schmalz, F.
1937 An explanation of the effect of heat treatment on wheat germ. Cereal Chem. **14**: 489–495.
Woodward, G. E., and Fry, E. G.
1932 The determination of blood glutathione. J. Biol. Chem. **97**: 465–482.

THE USE OF DIELECTRIC MEASUREMENTS TO DETERMINE THE MOISTURE CONTENT OF POWDERY SUBSTANCES. II

V. B. YEVSTIGNEYEV [1]

(Received for publication April 27, 1940)

In a previous article [2] were presented the results of experiments on the use of dielectric measurements to determine the moisture content of powdery substances. These results promised great possibilities of developing a perfectly satisfactory method suitable for practical application.

In order to avoid discrepancies in the results due to nonuniform grinding and density of packing of the substance in the condenser we applied a method of reducing the results to identical conditions by recalculating the experimental data according to a formula.

The best results, particularly for powdered biscuits, were obtained with the use of formula No. 1 deduced by the author on the basis of experimental data and Lichtenecker's logarithmic rule of mixtures:

$$\log E_1 = \frac{a_1^n \log E_2}{a_2^n}, \qquad (1)$$

where E_1 is the dielectric constant of the dielectric formed by the substance with which the condenser is filled up to a certain level (that is, a mixture of the substance and air) when the weight of the substance is a_1; E_2 is the dielectric constant of the same substance when the weight

[1] Most of the experimental work was carried out by M. A. Frolova.
[2] V. B. Yevstigneyev, The use of dielectric measurements to determine the moisture content of powdery substances, Cereal Chem. **16**: 336–353, 1939.

used to fill the condenser up to the same level is a_2; and n is an exponent $= 2/3 = 0.6667$.

If the dielectric constant is known for any given weight of the substance in the condenser, this formula may be used to determine the dielectric constant for any other weight of substance in the condenser. (It is to be understood that the condenser is always filled to the same volume.)

In the present work we give more detailed data obtained during verification of the method developed, together with further methodological development of this means of determining the moisture content of powdery substances from their dielectric properties. We worked with the same powdery substances as before, namely powdered biscuit, flour, and granulated sugar. However, attention was mostly directed to work on biscuit since in the previous work the most reliable results had been attained with this substance.

Determination of the Curve of the Dielectric Properties of Biscuit as a Function of Moisture Content

The problem of determining the curve showing the dependence of the dielectric properties on the moisture content should be studied with the greatest care, since the accuracy of all the subsequent work depends primarily on the correctness with which this is carried out. Attention must also be directed to the choice of material for study and to the means of obtaining specimens of different moisture content.

If we have to deal with a powdery substance with approximately identical grain size and of identical chemical composition in which only the quantity of moisture varies, then the choice of material presents no difficulties. In this case it is possible to use any specimens, attention being directed only to the selection of samples covering the desired range of moisture content.

For most of the products encountered in food industries the problem is not quite so simple. For instance, if we have to deal with meal products we find that the term " biscuit " covers a large number of products which differ greatly from one another in their chemical composition and physical properties. This is a result of using different recipes, different qualities and types of flour, of difference in the quantity of fats, different admixtures, etc.

Even with products the composition of which does not vary so greatly and which depend considerably less on human choice, such as grain, flour and tea, the difference between various kinds may be very considerable. In these cases the chemical composition and physical

properties of a single substance may vary appreciably, depending on the conditions of growth, weather, soil, climate, etc.

Differences of this kind are important because the results of measurements of the dielectric properties depend to a considerable degree on the chemical composition of the dry residue; moreover the influence of this is evident not only through changes in the quantity ratio of substances with different dielectric constants, but also through changes in the dielectric losses, in certain cases depending on the state of the water in the substance.

The second case is usually encountered on change in the ash content of the food product and the third on change in the quantities of component parts associated with water, for instance colloids and, particularly in the case of flour and dough, the quantity of gluten. Changes in chemical composition influence the results to a greater or less extent, depending on their size and kind, and are of an individual character for each product. It is therefore necessary to exercise great care and to take into account all the circumstances in choosing the material with which to determine the calibration curve for a given product. It is of course best, in view of these considerations, to specialize the calibration curve as far as possible, and to determine it for only one type of food product with the most constant composition of the dry substance.

In cases where this procedure is altogether impossible it is necessary to approximate it as closely as possible and to determine the curve for, say, a group of different varieties of the one product. For instance our experience shows that the differences between the physical-chemical properties of various kinds of sugar biscuits manufactured by the Moscow factories are not serious enough to prevent the consideration of " sugar biscuits " as an approximately uniform group. It is of course obvious that the moisture content of biscuits is such that comparatively small changes in the ash content should have no appreciable influence on the value of the losses. Differences in the chemical composition of the dry residue of the biscuit may be of inconsiderable influence owing to the small difference between the dielectric constants of the component parts. In these types of biscuits the gluten effect is largely overcome by the large quantities of sugar and fats so that changes in gluten content do not play a basic part. Finally, differences in structure are largely eliminated by the grinding process. As a result of these circumstances we were able to draw up a single curve for sugar biscuits, without subdividing the group according to the name of the biscuit and the place of manufacture.

The problem of obtaining specimens with different moisture contents necessary for the determination of the curve must be approached with care. There are two conceivable ways of doing this. First we

may select one specimen of the product which is most typical of the entire group in composition and alter its moisture content artificially. With this method it is easily possible to obtain a smooth curve with the defect, however, that it does not always express sufficiently well the relationship for the entire group. The method of varying the moisture content of the initial specimen is of great importance, at any rate in the case of powders. The least satisfactory method is to add different quantities of distilled water to a weighed amount of the initial substance. In this case, even if the subsequent mixing is very carefully carried out there is danger of nonuniform distribution of the water throughout the mass of the product. This in itself causes inaccurate dielectric measurements, since the results of these depend not only on the quantity of the substance but also on its spatial arrangement. In the case of ground biscuits the best results may be obtained by keeping the specimen over water in a closed space and mixing it constantly. This requires considerably more time, and again does not immediately lead to the requisite uniform distribution of moisture content. The specimens of increased moisture content which are obtained must be mixed very carefully and allowed to stand in a hermetically sealed vessel for two to three days before commencing the measurements. It is obvious that hygroscopic moisture cannot immediately penetrate quite uniformly throughout the entire thickness of the biscuit particles. Favorable conditions for obtaining a uniform distribution of water are afforded when one uses not simply hot water but certain saturated solutions of salts, which ensure a definite relative humidity of the air. The absorption of moisture will then proceed until the establishment of equilibrium between the vapor pressures above the surface of the biscuit and above the solution used, which occurs only when the moisture is uniformly distributed throughout the biscuit. However, the application of these solutions is more complicated and the total time required for complete absorption is not less but is actually considerably greater than with absorption over water.

The method of obtaining the curve by artificial variation of the moisture content is quite satisfactory and is even preferable to other methods for a preliminary investigation of the question as to whether there is a relationship between the moisture content of a substance and its dielectric properties. If one and the same specimen of the product is used for all the measurements, the influence of all other factors except the moisture content is excluded. However, in our opinion a more practical way of obtaining the curve is to take specimens of biscuit at different times from the factory and the shops with the object of obtaining products of different moisture content. A much greater number of specimens for investigation is needed with this method since the points, when plotted, do not usually lie on a smooth curve, as is almost always the

case when the moisture content of a single specimen is altered artificially. This effect, as previously noted, is primarily due to differences in the composition, structure, and similar properties of the specimens of different origin and composition.

The curve which is determined in this case nevertheless expresses the relationship between the dielectric constant and the moisture content in a manner which is much more likely to be correct for all types of sugar biscuit than when a single specimen is moistened artificially. This is because such a curve will characterize a certain sort of biscuit with average properties for the given group, and it is clear that the greater the quantity and variation in quality of the specimens investigated the more the curve obtained will be characteristic of the entire group, even though it is not altogether accurate for each individual case. The amount of scattering of the experimental points shows the validity or the inadvisability of uniting the chosen products into a single group. In choosing specimens for this method it is therefore necessary that the requisite variety of types of biscuit as well as different moisture contents be obtained.

The methods of preparing the specimens for analysis, of grinding them into a powder, of determining their moisture content, and of taking into account the density of packing may be varied at will with either of the methods described, but once these have been chosen for a given series of measurements they should be maintained unaltered until the series is completed.

Here it is necessary to mention the importance of the temperature at which the measurements are carried out. If the proper correction is not determined then the temperature must be allowed to vary as little as possible, say to less than $\pm$ 2°C., in order to avoid large errors. The results of experiments confirming this assertion will be shown later.

Experimental Procedure

In experiments which were carried out with the object of developing and verifying the method of determining the moisture content of biscuits from the dielectric constant, measurements were made at a temperature of 20 $\pm$ 2°C., using 25 specimens of biscuits obtained from the factory and from shops.

These specimens were ground with a mortar and pestle (but not too finely, in order to avoid unnecessary difficulties in the work) and were mixed in a vessel with a ground-glass stopper. The moisture content of the specimens was determined by the oven drying method, keeping them at a temperature of 130° C. for 40 minutes, which is taken as a standard in the manufacture of biscuits.

The ground specimen of biscuit was poured into the condenser up to a certain mark.[3] The method of filling the condenser with powder is not of great consequence; however the more uniformly and evenly the initial filling is carried out the better the agreement between repeated measurements. In our experiments we usually poured the powder into the condenser through a wide-mouthed funnel right up to the top of the condenser and then packed it down by gentle tapping and finally filled the condenser up to the mark with powder, packing it down in the same manner. Coincidence between the level of pouring and the mark on the condenser was determined by the eye. When the diameter of the upper part of the vessel is small only a slight error will occur. The filled condenser was immediately placed in the instrument and the capacitance reading was made, using the "Dielkometer" as described previously. Following this, the condenser was weighed to the nearest 0.1 g., to obtain the weight of the sample.

At the beginning of the experiment a control measurement was made with the condenser empty. With identical experimental conditions the reading should always be the same. In the event of small deviations due to differences in temperature or to fall in the accumulator voltage, a correction may be introduced.

We used this method of measurement for the preliminary and for the first verificative investigations; all the other measurements were carried out by a second method in which the fine condenser only was taken as the basis. For this method, at the beginning of a measurement the fine condenser scale was set at 90, the empty measuring condenser was placed in the circuit, and a reading was found with the rough tuning. This was taken as the zero position and the tuning of the rough condenser was then left unaltered. After the powder to be investigated had been poured up to the mark the reading was found by adjusting the scale of the fine condenser and this reading was taken as the value of the measurement.

The reading obtained by one method or the other is of purely arbitrary magnitude which depends both on the individual peculiarities of the instrument and on the capacities of the measuring and standard condenser. These readings may be used directly only to construct a curve either for powders which always fill the condenser in the same manner or for rough orientational measurements, since the dielectric constant of a powdery dielectric depends on the ratio of the volume occupied by the actual particles of powder to the volume of the spaces between them, that is, on the packing density.

In order to avoid the influence of packing density it must either be equalized or taken into account in some way. Generally speaking it is

[3] For a description of the measuring condenser see Cereal Chem. 16: 342.

possible to find experimentally the dependence of the instrument readings on the density of packing, characterizing the latter for instance by the weight of substance which is in the condenser. However in order to include all the possible cases of sample weight variation for different moisture contents in sufficient number for the determination of curves it is necessary to carry out a very large number of measurements. It is more convenient to recalculate the values and to relate them to a certain weight, as we did, since a much smaller number of specimens are required. The formula used for these calculations was given in the early part of the present paper. The weight to which the measurements are to be related may be chosen arbitrarily, but it is of course necessary to choose a suitable value and to avoid referring the data to a weight which cannot be put into the condenser.

We usually referred the results of our measurements to 100 g. simply because the condenser which we used holds approximately this quantity of biscuit. For reference to 100 g. formula No. 1 assumes the following form:

$$\log E_{100} = \frac{100^{2/3} \log E_2}{a^{2/3}} = \frac{21.54 \log E_2}{a^{2/3}}.$$

It is obvious from the notation that the results of the measurements should be substituted into the formula in the form of the dielectric constant (E_2) of the powder poured into the condenser. The reading obtained in scale divisions cannot be applied directly. Therefore, prior to the measurements, it is necessary to calibrate the variable condensers in terms of the dielectric constant under the same conditions as will be applied in the subsequent work. The simplest method of calibration is to use fluids of known dielectric constant, which are consecutively poured into the measuring condenser, plotting the condenser readings obtained on a graph.

The liquids used for the calibration can be prepared very simply by mixing two organic liquids, one of which has a lesser and the other a greater dielectric constant than that which is required for the calibration. This measure is, however, advisable only if it is possible to make an accurate measurement of the dielectric constants of the mixtures thus prepared. If this is not possible, then we may use pure organic liquids the dielectric constants of which are known for definite conditions of measurement.

We generally used the following pure liquids:

Petroleum ether	dielectric constant	1.88	at 20°C.
Cyclohexane	dielectric constant	2.005	at 20°C.
Benzene	dielectric constant	2.28	at 20°C.
Toluol	dielectric constant	2.38	at 20°C.

and also mixtures of toluol and petroleum ether, and toluol and 96% ethyl alcohol. On mixing small quantities of ethyl alcohol with toluol it was found necessary to add a little dioxane (diethylene dioxane, dielectric constant 2.23) in order to remove the turbidity due to the separation of water.

It should be mentioned that for practical purposes very great accuracy is not necessary in making the measurements of the absolute value of the dielectric constant of biscuit. If the error is systematic, *i.e.*, if the standard and measuring condensers are of sufficient mechanical strength and the scale reading always corresponds to the calibration, then a difference between the measured value of the dielectric constant and the true value of some 0.02 to 0.03 will not be of vital importance. Particular attention need be directed only to the identity of the calibration liquids on making a repeated calibration, a verification, or a transition from one condenser or instrument to another. The problem of these cases will be considered below. Particularly careful purification of the calibrating fluids using very laborious methods is therefore not justified in practice.

With each of our 25 specimens of biscuit the dielectric constant was measured and recalculated to 100 g. five times. The data obtained for 100 g. were plotted, the moisture contents being laid out as abscissas and the calculated values of the dielectric constants as ordinates. The curve is shown in Figure 1. This curve is of the same character as

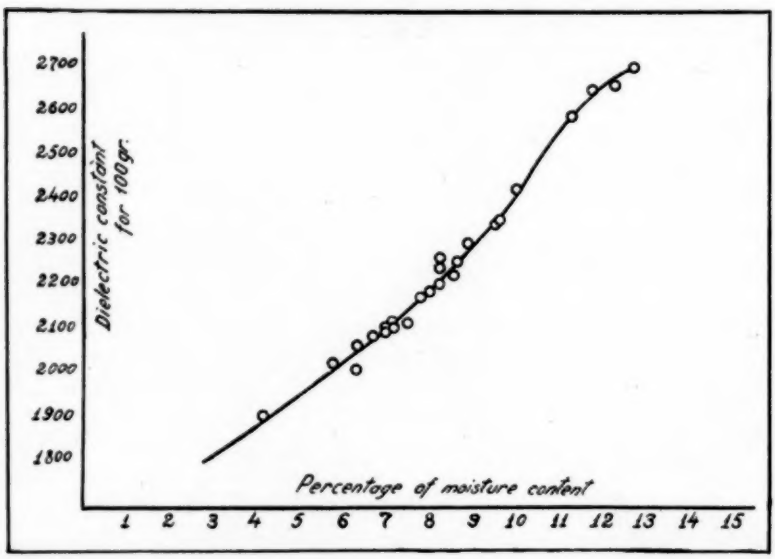

Fig. 1. Curve of the dielectric constant of sweet biscuit as a function of moisture content for a weight of 100 grams.

that obtained in the preliminary experiments; it rises at first slowly and then more rapidly as the moisture content is increased. The upper bend cannot be considered as altogether reliably established owing to its position and to the small number of experimental points through which it is drawn. This curve may be used for practical determinations of the moisture content of biscuit if the latter is ground, poured into the condenser, weighed, and if the dielectric constant obtained is recalculated to refer to 100 g. However the necessity for recalculation, which incidentally is quite complicated, is of great inconvenience for practical work and requires considerable time and a certain degree of experience. In order to avoid this objection and to render the method truly rapid we decided to draw on the same co-ordinates a curve of the dependence of the dielectric constant on the weight of biscuit. The problem therefore consists in constructing a graph to express a function of three variables, the moisture content, the dielectric constant, and the weight of biscuit. For this purpose we chose the method of constructing a family of curves of dielectric constant vs. moisture content with the biscuit weight serving as a parameter characterizing the individual curves. If the weight is taken as the parameter which varies by steps, the necessary family of curves may be constructed by plotting, along with the curve relating to 100 g., curves for other weights.

It is inadvisable to calculate the data for these curves from the initial measurements on the specimens of biscuit substituting another weight into the formula in place of 100 g. It is much more convenient, proceeding from the curve already plotted (Fig. 1), to mark on it a number of points, read off the corresponding moisture content and dielectric constant, and then recalculate the data for the corresponding weights. We carried out this recalculation for 105, 110, 115 g. and so on up to 140 g. with 5-g. intervals. In order to recalculate for 105 g. the following numerical values are substituted into formula No. 1:

$$\log E_{105} = \frac{105^{2/3} \log E_{100}}{100^{2/3}} = 1.0334 \log E_{100}.$$

The factor 1.0334 remains constant for all values of the dielectric constant (E_{100}). Consequently the recalculation is quite simple. The factors obtained in this way for recalculation to 110, 115 g. etc., are given in Table I.

The moisture content and dielectric constants corresponding to the points taken on the curve for 100 g. and the data after recalculation to the various weights are given in Table II. In order to simplify somewhat the construction of the curves the points were chosen with moisture-content intervals of 1%.

TABLE I

CALCULATIONS FOR SAMPLES OF VARIOUS WEIGHT

Weight to be recalculated	Factor
g.	
105	1.0334
110	1.0659
115	1.0980
120	1.1295
125	1.1606
130	1.1913
135	1.2219
140	1.2521

TABLE II

DIELECTRIC CONSTANTS FOR VARIOUS WEIGHTS

Moisture content	100	105	110	115	120	125	130	135	140
%	g.	g.	g.	g.	g.	g.	g.	g.	g.
3	1,810	1,846	1,882	1,919	1,955	1,989	2,028	2,065	2,102
4	1,870	1,910	1,949	1,988	2,028	2,068	2,108	2,149	2,190
5	1,943	1,987	2,030	2,074	2,118	2,162	2,206	2,252	2,297
6	2,020	2,068	2,116	2,164	2,213	2,261	2,311	2,361	2,412
7	2,105	2,158	2,211	2,264	2,318	2,383	2,427	2,483	2,540
8	2,200	2,259	2,317	2,377	2,437	2,497	2,558	2,621	2,684
9	2,310	2,376	2,441	2,508	2,574	2,643	2,711	2,782	2,853
10	2,440	2,514	2,588	2,663	2,739	2,816	2,894	2,974	3,055
11	2,590	2,674	2,758	2,843	2,930	3,018	3,107	3,191	3,292

These data were used in the construction of the family of curves illustrated in Figure 2. There is an individual curve for each of the weights for which the recalculation was carried out. The curves for intermediate weights may easily be obtained by simple graphical interpolation.

To effect a final simplification of the work of transition from dielectric measurements to moisture content by means of these curves, additional notation was introduced into the latter. First, in order to avoid the necessity of recalculating the reading of the " Dielkometer " to the absolute value of the dielectric constant we have laid out on the ordinate, together with the values of the dielectric coefficients, the corresponding scale indications of the instrument. The figures are taken from the calibration curve for the given measuring condenser. Finally, at the upper ends of the curves we have given the weight of the biscuit plus that of the condenser. This avoids the necessity of subtracting the weight of the empty condenser each time. As a result of these various measures the process of determining the moisture content from the dielectric constant in the case of ground biscuit requires very little time.

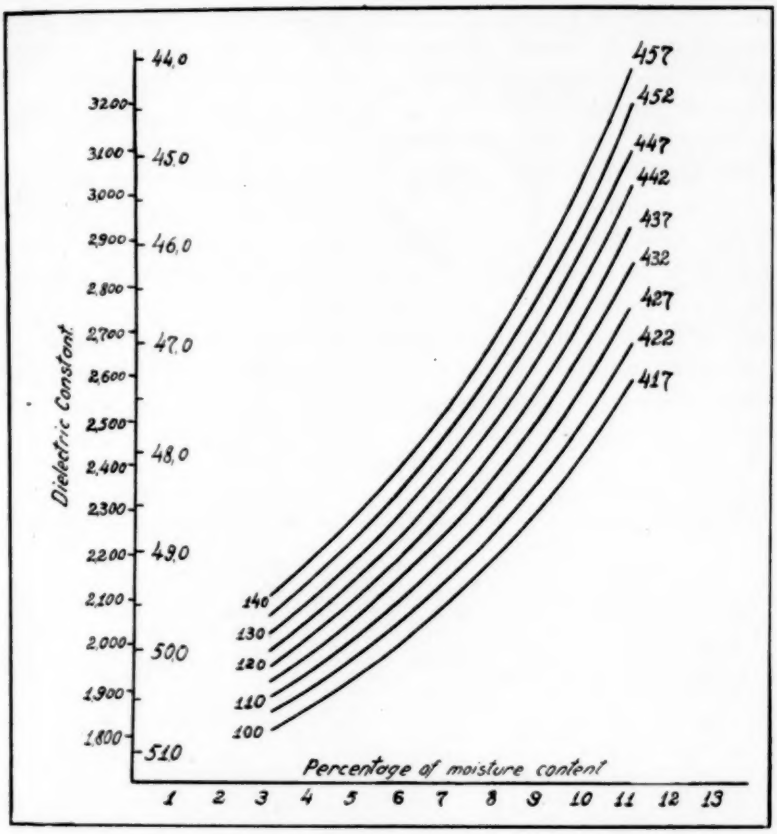

Fig. 2. Curve of the dielectric constant of biscuit as a function of moisture content for different weights.

To carry out a determination it is only necessary to pour the ground biscuit into the condenser up to the mark, pack it down somewhat by tapping the condenser, insert the condenser into the instrument to take the reading, and weigh the condenser on a balance with an accuracy of 0.3–0.5 g. From the point on the ordinate which corresponds to the "Dielkometer" reading obtained it is then necessary to draw a line parallel to the abscissa and on the curve corresponding to the appropriate weight of biscuit to read off the corresponding moisture content on the abscissa. This can all be done in 3 to 5 minutes.

Control Experiments

In order to determine to what extent the foregoing method is practically applicable, to find the degree of reproducibility when using the family of curves for different weights, and to find the accuracy with

which the moisture content was determined, we carried out control measurements of moisture contents of 20 specimens of sugar biscuits simultaneously by means of a drying oven and the " Dielkometer." The method of carrying out the measurements was the same as in determining the curves. The data are given in Table III.

The arithmetical mean of the average divergences between the results of the measurements obtained from the dielectric constant and those obtained with the drying oven is 0.14% of moisture. The average deviation in each case does not, as a rule, exceed 0.15% to 0.20% of moisture. There were, actually, only two exceptions to this. The divergences between repeated determinations of the moisture content from the dielectric constant do not, in most cases, exceed 0.15% to 0.20%. Taking into account the rapidity of the analysis, these results may be

TABLE III

APPLICATION OF THE METHOD TO 20 BISCUIT SAMPLES

Exp. No.	Exp. temp.	Moisture content, desiccation method	Dielectric const. from instrument reading	Weight held by condenser	Dielectric const. referred to 100 g.	Moisture content, from dielectric const. curve		Divergence	Divergence mean of 3 results
	°C.	%		g.		%	*Mean*	%	%
1	22°	6.82	2.477	138.0	2.08	6.75	—	−0.07	—
2		6.82	2.435	133.3	2.09	6.85	6.78	+0.03	−0.04
3		6.82	2.444	134.9	2.08	6.75	—	−0.07	—
4	22°	8.07	2.531	129.5	2.18	7.85	—	−0.22	—
5		8.07	2.545	128.4	2.20	8.10	7.98	+0.03	−0.09
6		8.07	2.556	130.7	2.20	8.10	—	+0.03	—
7	22°	8.45	2.663	131.7	2.26	8.50	—	+0.05	—
8		8.45	2.658	132.4	2.25	8.50	8.48	+0.05	+0.03
9		8.45	2.636	131.2	2.24	8.45	—	−0.00	—
10	19°	12.34	2.991	117.3	2.68	11.70	—	−0.64	—
11		12.34	2.982	116.5	2.68	11.70	11.73	−0.64	−0.61
12		12.34	3.041	117.0	2.72	11.80	—	−0.54	—
13	19°	7.88	2.553	128.5	2.21	8.15	—	+0.27	—
14		7.88	2.510	126.1	2.20	8.00	8.05	+0.12	+0.17
15		7.88	2.523	127.6	2.20	8.00	—	+0.12	—
16	19°	11.44	2.969	114.5	2.70	11.70	—	+0.26	—
17		11.44	2.934	114.9	2.66	11.50	11.58	+0.06	+0.14
18		11.44	2.910	113.2	2.67	11.55	—	+0.11	—
19	17.5°	5.97	2.335	139.0	1.98	5.55	—	−0.42	—
20		5.97	2.335	136.6	1.99	5.60	5.58	−0.37	−0.39
21		5.97	2.324	136.0	1.99	5.60	—	−0.37	—
22	18°	9.13	2.630	125.4	2.30	8.90	—	−0.23	—
23		9.13	2.619	124.3	2.30	8.90	8.93	−0.23	−0.20
24		9.13	2.682	127.2	2.32	9.00	—	−0.13	—
25	19°	9.72	2.706	121.1	2.40	9.75	—	+0.03	—
26		9.72	2.754	123.5	2.41	9.80	9.78	+0.08	+0.06
27		9.72	2.774	124.4	2.41	9.80	—	+0.08	—
28	19°	8.10	2.553	128.8	2.21	7.95	—	−0.15	—
29		8.10	2.553	126.9	2.22	8.15	8.08	+0.05	−0.02

TABLE III—(*Continued*)

Exp. No.	Exp. temp.	Moisture content, desiccation method	Dielectric const. from instrument reading	Weight held by condenser	Dielectric const. referred to 100 g.	Moisture content, from dielectric const. curve		Divergence	Divergence mean of 3 results
	°C.	%		g.		%	*Mean*	%	%
30		8.10	2.545	126.3	2.22	8.15	—	+0.05	—
31	20°	6.01	2.324	129.9	2.03	6.10	—	+0.09	—
32		6.01	2.341	130.5	2.04	6.25	6.23	+0.24	+0.22
33		6.01	2.357	130.8	2.05	6.35	—	+0.34	—
34	20°	6.70	2.378	129.8	2.07	6.70	—	0.00	—
35		6.70	2.435	132.1	2.09	6.90	6.77	+0.20	+0.07
36		6.70	2.361	127.7	2.07	6.70	—	0.00	—
37	20°	5.50	2.269	130.9	1.98	5.55	—	+0.05	—
38		5.50	2.269	129.9	1.99	5.60	5.58	+0.10	+0.08
39		5.50	2.269	130.3	1.99	5.60	—	+0.10	—
40	20°	6.78	2.313	122.4	2.08	6.75	—	−0.03	—
41		6.78	2.324	123.3	2.08	6.75	6.77	−0.03	−0.01
42		6.78	2.322	122.3	2.09	6.80	—	+0.02	—
43	20°	6.44	2.313	124.0	2.07	6.65	—	+0.21	—
44		6.44	2.304	124.4	2.06	6.55	6.62	+0.11	+0.18
45		6.44	2.313	123.3	2.07	6.65	—	+0.21	—
46	20°	7.85	2.444	118.4	2.22	8.10	—	+0.25	—
47		7.85	2.424	117.2	2.22	8.10	8.05	+0.25	+0.20
48		7.85	2.378	115.0	2.20	7.95	—	+0.10	—
49		7.80	2.444	121.7	2.19	7.85	—	+0.05	—
50	20°	7.80	2.438	121.3	2.19	7.85	7.93	+0.05	+0.13
51		7.80	2.490	123.5	2.21	8.10	—	+0.30	—
52	20°	7.71	2.400	119.8	2.17	7.70	—	−0.01	—
53		7.71	2.413	121.1	2.17	7.70	7.70	−0.01	−0.01
54		7.71	2.398	119.8	2.17	7.70	—	−0.01	—
55	19°	7.86	2.400	117.6	2.19	7.95	7.90	+0.09	+0.04
56		7.86	2.422	120.6	2.18	7.85	—	−0.01	—
57	19°	8.49	2.575	124.5	2.26	8.60	—	+0.11	—
58		8.49	2.545	122.6	2.26	8.60	8.57	+0.11	+0.08
59		8.49	2.551	123.8	2.25	8.50	—	+0.01	—

considered as quite satisfactory and the "lightning" method of determining the moisture content from the dielectric constant is of quite sufficient accuracy for practical use in manufacturing control of the moisture of biscuits.

Further Development of Method

Although the results of the experiments described above permit of the conclusion that the determination of the moisture content of biscuit from the dielectric constant is already far enough developed to be applicable for practical purposes, it is nevertheless not as yet sufficiently standardized. According to the method herein presented an individual curve of the dependence of the dielectric properties of the biscuit as a function of the moisture content must be drawn up for each instrument

and each measuring condenser. The experimenter is limited to that condenser for which the curve is already determined. If it is necessary to replace the condenser by one of another shape or size, or if the instrument becomes damaged or if the plates of the old condenser are renewed, all the previous work must be repeated from the very start. This is a great inconvenience which may sometimes lead to complications in the work. Further, this circumstance might serve as an obstacle to extensive use of the method, since in production laboratories there is not always available a personnel sufficiently qualified to draw up an accurate calibration curve and to check on its reliability during subsequent use. It would be much more convenient if for a given type of product there were available a curve or system of curves established at some center and which were applicable for all cases of determination of the moisture content of this product from the dielectric constant. Such a curve might be obtained and used either through the availability of absolutely identical instruments and condensers, or by the development of a method of translation of results from one condenser to another. The manufacture of absolutely standard apparatus is so difficult that we considered it necessary to develop the method described below, which is based on principles already previously discussed.

A complicated question, for practical purposes, is the determination of the correction for differences between the temperature at which the calibration was carried out and that at which the measurement takes place. In conditions of manufacture it is not always possible to carry out the measurements at a temperature which is very close to the temperature of calibration. A large deviation in temperature may introduce a very considerable error into the moisture content determination.

Furthermore, it is very desirable to simplify the calculation of the measurement of the dielectric constant to one and the same packing density in the determination of the initial curve. Since we carried out all the subsequent work using a simpler method of calculation it is desirable to begin our further discussion of the method with this feature.

Simplification of the Calculations for Drawing Up the Calibration Curve

Examination of formula No. 1 shows that the dielectric constant (E_1 and E_2) stands under the logarithmic sign on both sides of the equation. Hence it is clear that there will be rather less calculation to carry out if as the basic magnitude we take not the dielectric constant (E) but its logarithm to the base 10 ($\log E$). The substitution should be introduced at the very start, from the drawing up of the curve for transition from the readings of the " Dielkometer " to the absolute value of the

dielectric constant with a given measuring condenser as determined from the calibrating liquids.

Instead of plotting as ordinates the values of the dielectric constants of these liquids, the *logarithms* of their dielectric constants should be plotted. Figure 3 is an example of such a curve for the condenser with which our basic work was carried out. The corresponding value of log E may be found from this curve for any reading of the standard condenser. The value of the logarithm which is found is substituted

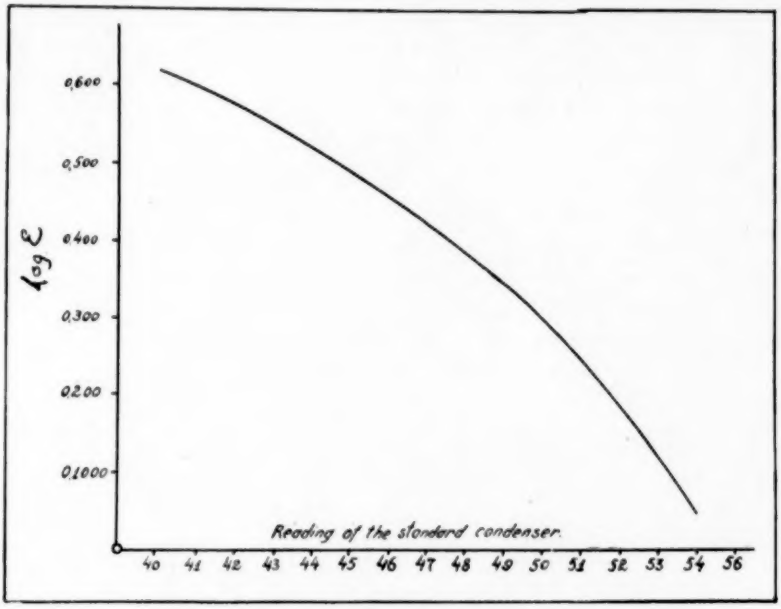

Fig. 3. Calibration curve for log E.

directly into formula No. 1. Using this method the number of times necessary to take logarithms is reduced by one.

If $L_1 = \log E_1$ and $L_2 = \log E_2$, then formula No. 1 assumes the following form:

$$L_1 = \frac{a_1^{2/3} L_2}{a_2^{2/3}}.$$

From the recalculation we obtain the logarithm of the dielectric constant corresponding to the weight to which all the data must be referred and thus avoid an expenditure of time in finding the number from its logarithm. Curves of the dependence of the logarithm of the dielectric constant on the moisture content of biscuit for different weights should be drawn up in the same way as before.

The recalculation becomes more simple if the entire work of calculation is replaced by the use of nomograms. We drew up a simple nomogram as shown in Figure 4 for referring the dielectric constant of biscuit for weights of 80 to 140 g. to a weight of 100 g. Certain changes were introduced into the basic formula for the purpose of drawing up this nomogram. The value of n in formula No. 1 was taken as 0.7 instead of 2/3. Experience shows that in this case the difference between the various weights of samples is perhaps even better than when we used $n = 2/3$.[4] The absolute values of the dielectric constant are somewhat altered but the calculation of nomograms becomes simpler.

Since almost all the experiments described below were carried out with the aid of nomograms it is necessary to bear in mind that the absolute values obtained in these experiments are incomparable with those obtained in the previous ones.

The formula was converted in the following manner for the purpose of drawing up the nomograms:

$$\frac{\log E_1}{\log E_2} = \frac{a_1^{0.7}}{a_2^{0.7}},$$

or

$$\log \log E_1 - \log \log E_2 = 0.7 \log a_1 - 0.7 \log a_2,$$

and for $a_1 = 100$ g., $\log \log E_1 - \log \log E_2 = 1.4 - 0.7 \log a_2$. The equation thus obtained affords the simplest means of obtaining relations between the three magnitudes E_1, E_2, and a_2 by means of three parallel scales. The method of constructing these nomograms may be found in any textbook on the construction of nomograms. In accordance with the usual method for using nomograms the measured value of the dielectric constant read off on the left-hand scale is connected by a straight line with the value corresponding to the weight on the right-hand scale, and the intersection of this line with the center scale gives the required value of E for 100 g.

Even though our nomogram was primarily intended for recalculation of the weights to 100 g., its applicability is not limited to this. From the relationship:

$$\frac{\log E_1}{\log E_2} = \frac{a_1^{0.7}}{a_2^{0.7}} = \left(\frac{a_1}{a_2}\right)^{0.7},$$

it is clear that the absolute values of a_1 and a_2 play no part and it is immaterial whether the value of the dielectric constant is recalculated from 80 g. to 100 g. or from 40 g. to 50 g., etc., provided the ratio of the values is constant. Therefore, on the right-hand scale of the nomogram we may replace the value of 100 g. by any other number, for

[4] We verified the applicability of formula No. 1 with $n = 0.7$ also for determining the moisture content of black tea, which enlarges the field for its use.

instance 20, while the remaining scales increase or decrease in the same proportion as the new number compares with 100. In this example it would be 100/20 equals 5. The nomogram may then be used to recalculate the data to the new weight (20 g.) instead of to 100 g.

Further, the nomogram may be used for the reverse recalculation from 100 g. to any other weight from 140 to 80 g., which is necessary for instance in drawing up the systems of curves in Figure 2. For this purpose the weight for which the recalculation is to be made is taken on the right-hand scale, the dielectric constant for 100 g. on the center scale, and the desired value of the dielectric coefficient is read off the left-hand scale.

Transition from One Condenser to Another

The final result of the measurement of the dielectric properties of powdery substances by our method is determined by two factors, the dielectric constant as obtained from the reading of the instrument, and the correction for variations in the density of packing. Therefore, on transition from one condenser to another it is necessary, in order to obtain comparable results from the measurements, that comparable results be obtained on measuring each of these factors individually. Identity of purely electrical measurements of the dielectric constant may be obtained by calibrating the condensers under perfectly identical conditions with calibrating liquids at a given temperature.

The following points must be taken into account in considering the density of packing: In the method which we have used the density of packing is governed by the weight of the substance required to fill the condenser to a given volume. This method, which is the simplest and most convenient for practical purposes, is applicable only to each condenser individually, since the weight of the substance required to fill the condenser depends on the size of the vessel in which the plates are fixed. If the density of packing is constant the quantity of substance which the condenser will hold is directly proportional to its volume. Therefore, on transition from one condenser to another it will primarily be necessary to take into account the ratio of the volume for holding powder in the one condenser to that in the other and to alter proportionately the constant weight to which all the results are referred. Suppose for instance that it is necessary to compare the density of packing in two condensers, one of which is of a volume of 237 cc. and the other 185 cc. If in the case of the first condenser all the results were referred to 100 g., then to obtain comparable results with the second condenser the data should be referred to a weight of

$$\frac{100 \cdot 185}{237} = 78.06 \text{ g.}$$

While this method is quite suitable for practical purposes, nevertheless it is more convenient to carry out the recalculation always to one and the same weight. For this purpose it is necessary to introduce a correction, not in the standard weight, but in the weight of powder which is poured into the condenser, which must be adjusted in accordance with the change in volume. If in the above example it is desired to effect the reference to 100 g., one should proceed in the following manner: Suppose that 69.4 g. of the investigated powder are required to fill the second condenser. We will calculate the weight of powder which would have been required to fill the first condenser to the same packing density. The volume of the first condenser is $237/185 = 1.281$ times greater than the volume of the second condenser. Consequently the weight required to fill the condenser with the same density of packing must be multiplied by the same factor, *i.e.*, $69.4 \times 1.281 = 88.9$ g. This is the weight which must be used in making the calculations.

This method has the advantage that the nomograms already prepared can be used for the final calculations of the dielectric constant. However the most general and simple method is to refer the results not to some definite weight required to fill the condenser up to a certain mark, but to the weight of powdery dielectric which occupies unit volume. The value obtained in this case characterizes the packing density uniquely, independently of the type of condenser. There can be no difficulty in calculating this value. Knowing the weight of powder and the volume of the measuring condenser and dividing the one by the other we easily obtain the desired result.

If a_1 and a_2 are weights required to fill the condenser to two different packing densities and v is the volume of the condenser, then formula No. 1 may be written:

$$\log E_1 = \frac{\left(\frac{a_1}{v}\right)^n \log E_2}{\left(\frac{a_2}{v}\right)^n} = \frac{p_1{}^n \log E_2}{p_2{}^n}, \qquad (2)$$

where p_2 represents the packing density obtained at the time of measurement and p_1 the packing density taken as standard.

The nomogram illustrated in Figure 4 may be altered to correspond with this. As the starting point we may take formula No. 2, which is transformed to:

$$\log \log E_1 - \log \log E_2 = 0.7 \log p_1 - 0.7 \log p_2.$$

The actual numerical value to which the results are necessarily referred in this case, as before, depends on the way the investigated substance packs into the condenser. In our condenser, with 100-g. packing den-

sity, the quantity of biscuit in unit volume (1 cc.) was 0.422 g. of powder. Hence, in order to deal with round numbers, the reference may be made to a packing density of, for instance, 0.45, which leads to the following formula for the nomogram:

$$\log \log E_1 - \log \log E_2 = 0.7 \log 0.45 - 0.7 \log P_2.$$

In order to be able to judge to what extent the described method of transition from one condenser to another is practical we measured the dielectric constant of biscuit simultaneously with two condensers of very different sizes. The first condenser was the same one as was used in the basic measurements; its volume was 237 cc. The second condenser was of similar design to the first, but its volume was only 62.7 cc.

The second method of reducing the results to comparable data was used. The weight of powder required to fill the second condenser was multiplied by $237/62.6 = 3.78$ and the calculation was then effected by means of the nomogram illustrated in Figure 4. Since these methods

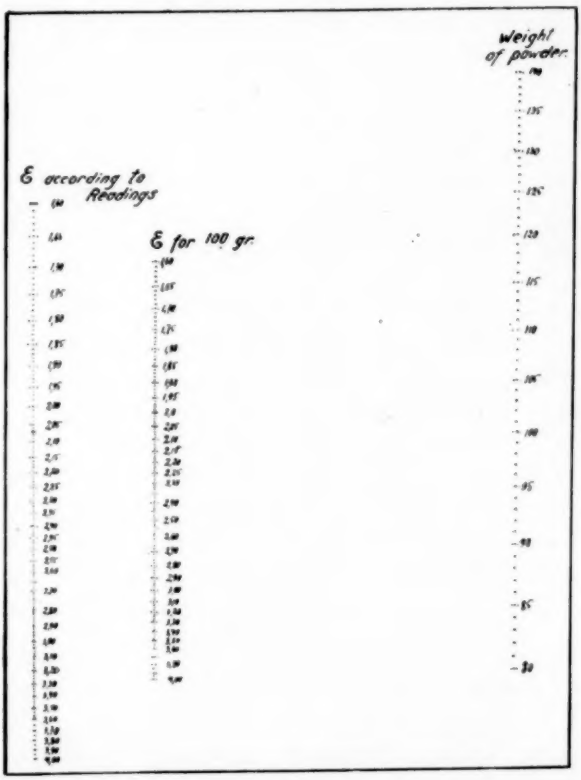

Fig. 4. Nomogram for recalculation of the dielectric constant of powder to a weight of 100 grams.

differ from each other only in the practical method of calculation, the results of the experiments should be representative of all of them. The calibration of the condenser was carried out with the same calibrating liquids as before at a temperature of 25°C. The biscuit was ground with a mortar and pestle, carefully mixed, and poured into the two condensers in turn. The measurements were carried out at different temperatures and with different specimen moisture contents. The numerical data are given in Table IV. It should be borne in mind that each figure is an average of five values obtained or calculated from experimental data.

TABLE IV

DETERMINATIONS WITH CONDENSERS OF DIFFERENT CAPACITY

			Condenser 1			Condenser 2				
No.	Temp.	Moisture content, drying cupboard	Mean weight to fill	Mean dielectric const., from instrument	Mean dielectric const., after reference to 100 g.	Mean weight to fill	Mean weight after multiplication by 3.78	Mean dielectric const., read off instrument	Mean dielectric const., after reference to 100 g.	Diff. (100 g.) 1st and 2nd condensers
	°C.	%	g.			g.	g.			
1	25	8.31	117.7	2.62	2.37	31.5	118.9	2.62	2.35	−0.02
2	25	6.05	111.3	2.25	2.12	29.2	110.3	2.24	2.13	+0.01
3	25	8.61	117.7	2.65	2.39	31.6	119.6	2.65	2.37	−0.02
4	25	7.35	116.8	2.43	2.21	31.1	117.2	2.43	2.21	−0.00
5	17	8.60	114.9	2.56	2.36	30.3	114.5	2.59	2.38	+0.02
6	24	9.37	114.9	2.72	2.49	30.2	114.3	2.74	2.51	+0.02
7	14	8.21	121.1	2.54	2.26	31.1	117.5	2.51	2.27	+0.01
8	15	7.16	119.8	2.37	2.15	31.5	119.2	2.37	2.15	0.00
9	15	6.17	112.2	2.17	2.05	26.4	100.1	2.06	2.06	+0.01
10	13	8.02	115.8	2.43	2.23	30.9	116.7	2.48	2.26	+0.03
11	14	8.02	119.7	2.49	2.24	32.0	121.1	2.55	2.26	+0.02
12	15	8.40	122.8	2.60	2.29	33.1	125.1	2.70	2.30	+0.01
13	16	7.61	121.8	2.48	2.22	32.5	123.7	2.56	2.24	+0.02
14	18	7.80	128.7	2.66	2.28	34.4	128.1	2.71	2.30	+0.02
15	20	10.80	114.9	2.98	2.70	31.1	119.1	3.05	2.71	+0.01
16	25	8.38	121.3	2.68	2.37	32.0	120.9	2.69	2.38	+0.01
17	25	8.12	117.7	2.55	2.31	31.8	120.3	2.61	2.33	+0.02
18	25	7.06	120.9	2.48	2.22	33.1	125.1	2.54	2.23	+0.01
19	20	8.61	123.1	2.69	2.36	33.8	127.7	2.75	2.35	−0.01

The difference between the dielectric constants referred to the same packing density does not, as a rule, exceed 0.01 to 0.02 units of dielectric constant and the method described for transition from one condenser to another may be considered as suitable for practical purposes. The same methods may also be applied when it is necessary to change over from one instrument to another.

The methods described make it possible to use curves of the moisture content of the substance (as a function of its dielectric properties) established at different laboratories with different instruments and condensers. For this purpose it is only necessary to calibrate the instrument with the measuring condenser using the calibrating liquids, which is far easier than it is to determine the entire curve. We again emphasize that particular attention should be directed to identity of the standard calibrating liquids and of the conditions of calibration in the two cases.

Influence of Temperature on the Results of the Measurements

Investigators who have studied methods of using dielectric measurements to determine the moisture content of substances have noted the great influence of temperature on the results. In view of the inconvenience and often the impossibility of maintaining the required temperature under manufacturing conditions it is absolutely necessary to determine the correction for deviations in the temperature from the standard value. The influence of the temperature on the moisture determination is a total of the influences of the temperature on the measuring instrument and on the condenser and of the influence of the latter on the dielectric properties of the substance. Since our practical objective was to determine the value of the correction for our conditions, we were not interested in each of these individual factors, although it would be worth while to investigate each of them separately. Therefore, in working with biscuit, the temperatures of the instrument and the substance measured were altered simultaneously.

During the course of the experiments the "Dielkometer" was situated in a wooden thermostat which was insulated on the outside with asbestos. Heating was effected with an incandescent lamp, and the required temperature was maintained (by means of a thermoregulator) to an accuracy of ± 0.2°C. Uniformity of the temperature throughout the thermostat was attained by use of a table fan. Calibration of the condenser with the standards liquids was carried out at 25°C. Measurements on specimens of ground sugar biscuit of different moisture contents were carried out at temperatures of 15°, 20°, 25°, and 35°C.

In view of the possibility that their moisture content might change if the specimens were to be poured several times, the determination of the moisture in the drying oven was carried out before each series of measurements. The instrument, the measuring condenser, and the specimens of biscuit contained in vessels with ground-glass stoppers were maintained in the thermostat at the given temperature for not less than 10 to 12 hours.

The results of the measurements were plotted with the dielectric constants of the powders related to 100 g., using the formula with $n = 0.7$ laid out on the vertical axis and the moisture contents on the horizontal one. A curve for the relation of the dielectric constant of the biscuit to the moisture content was drawn in the usual manner through the experimental points for each of the temperatures used. The points obtained, together with the corresponding curves, are shown in Figure 5.

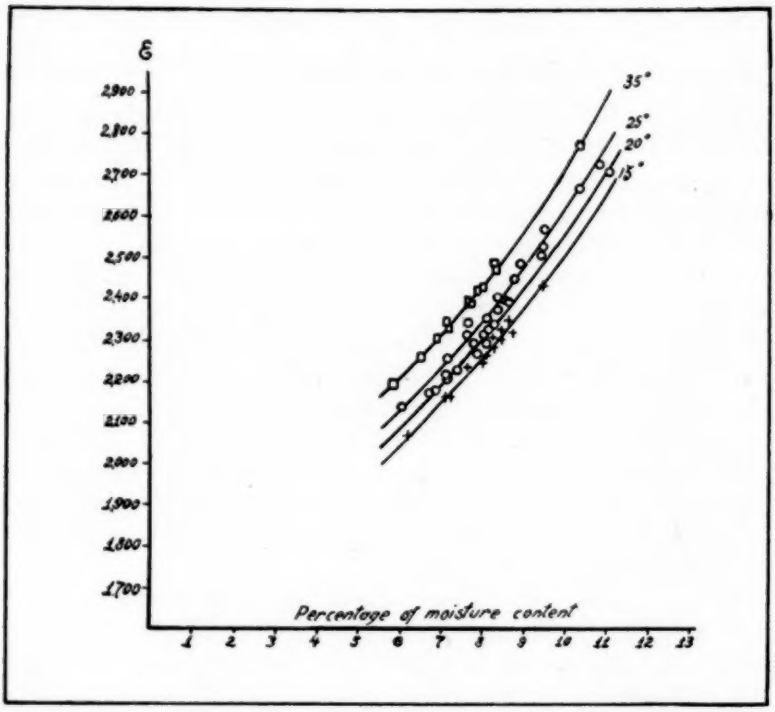

Fig. 5. Curve of the dielectric constant of biscuit as a function of moisture content under different temperatures.

There is observed a clear and regular dependence of the dielectric properties of the biscuit on the temperature. The higher the temperature of measurement the higher the position of the curves. In using these curves it is necessary to bear in mind that they express the total dependence of the instrument readings and of the substance properties on the temperature. Therefore, in principle, these curves are only suitable for practical application to the instrument for which they were drawn up and may be used only with a certain degree of approximation with other instruments of the same manufacture. For instruments

which are very different in design from the " Dielkometer " these curves may be altogether unsuitable.

Comparing the positions of the curves illustrated in Figure 5 it will be seen that when the temperature changes by 10°C. the dielectric constant of the powder changes by 0.08 to 0.11 unit, the temperature having a greater influence the greater the moisture content. This corresponds to an error in the determination of the moisure content of approximately 0.7 to 0.08%; *i.e.*, the correction per 1°C. is equal to about 0.07% to 0.08% of moisture content.

Concerning the Recalculation Formula

The main difference between the trend of our work and that of others who have studied problems of the determination of the moisture content of powders consists in the mathematical levelling of the packing density by means of an equation deduced on the basis of Lichtenecker's logarithmic law of mixtures:

$$\log E_1 = \frac{a_1^n \log E_2}{a_2^n}.$$

There are four magnitudes in the right-hand side of this equation of which three are obtained experimentally and one, n, is a constant.

It was found experimentally that the best agreement between results for different weights recalculated to 100 g. was obtained with n equal to between 0.65 and 0.7. There is little reason to suppose that the absolute value of n may be the same for other substances. Essentially, by changing the value of n which is the power to which the weight is raised, we attribute greater or less significance to the density of packing as one of the conditions which determine the dielectric constant of the powder. The graph given in Figure 6 shows how greatly this value changes. This graph shows the change in the value of the dielectric constant obtained from formula No. 1 with various values of n from 0 to 1 with weights of from 80 to 140 g. referred to 100 g., when the measured value of the dielectric constant is 2.0. The values of n are plotted on the abscissa and those of the dielectric constant on the ordinate. As n is increased the curves diverge increasingly. For instance if $n = 0.1$ and the weight of the biscuit is 120 g. recalculation gives a value of the dielectric constant of 1.975; with the same weight of biscuit and $n = 0.5$ the dielectric constant is 1.883; while for $n = 1$ the dielectric constant is 1.782. When $n = 0$ the weight plays no part at all.

Since the weight determines only the quantity of substance, not its quality and structure, it is in the present case a purely conditional magnitude which is easily measured in practice and its relative importance in

determining the value of the dielectric constant will obviously vary with alteration in the composition and properties of the powdery dielectric. A consequence of this is that it may be necessary to change the value of n for different materials in order to obtain the best results of recalculation. This fact is of immense importance for practical purposes. It may be supposed that one of the main factors which influence n will be the electrical conductivity of the powder which conditions the dielectric losses. We decided to verify this supposition in the following manner.

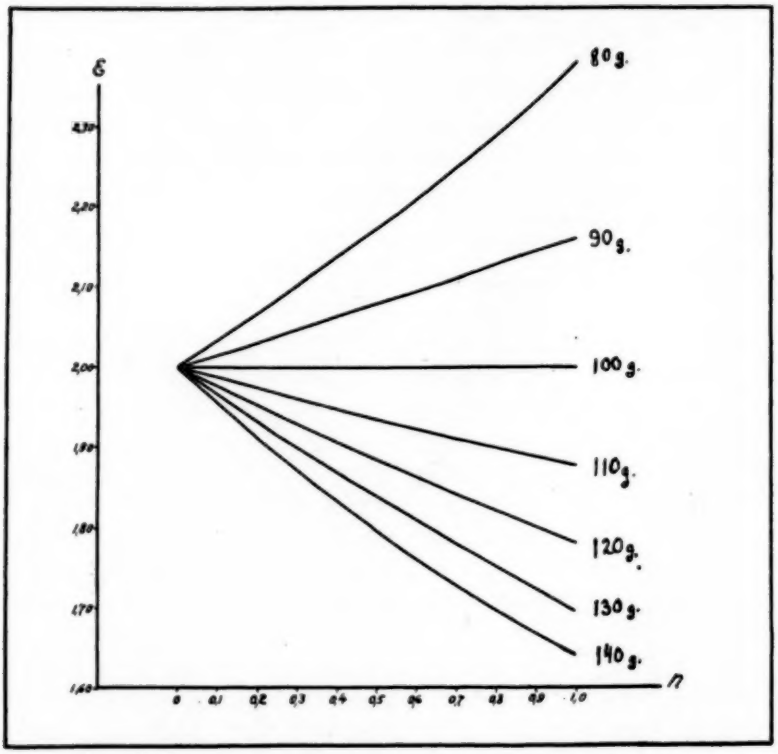

Fig. 6. Influence of the magnitude n on the results of the recalculation.

A specimen of tempered quartz sand, which had been very carefully washed free of all salts, was divided into several parts. Into each of these specimens we introduced standard quantities of KCl solution of different concentrations. It is clear that the greater the concentration of the KCl the greater the electrical conductivity of the mixture. The specimens of sand with different degrees of electrical conductivity thus prepared were poured into the measuring condenser by two methods: with very slight and with the densest possible packing which could be

obtained by shaking and tapping on the walls of the condenser. Readings were taken on the instrument in the usual manner, and afterwards the condenser was weighed. From the experimental values we calculated the value of n most suitable for recalculating the results.

The following equation for finding n was obtained by taking the logarithm of formula No. 1:

$$n = \frac{\log \log E_1 - \log \log E_2}{\log a_1 - \log a_2}.$$

Into this equation we substituted values for E_1 and E_2, a_1 and a_2, which were obtained from two measurements with one and the same specimen but with different densities of packing which were combined in such a way as to obtain the greatest possible difference between the densities of packing.

About 20 experimental measurements were taken for each test and several dozens of pairs of combinations were taken for calculation of the value of n. The arithmetic mean of the results obtained was then taken, and this was accepted as the most suitable value for n. The data are given in Table V.

TABLE V

VALUES OF n FOR SAND

Investigated substance	Average value n	Investigated substance	Average value n
Dry quartz sand	0.9906	Quartz sand with 0.02N KCl (3 cc. per 100 g.)	0.7089
Quartz sand with water (2 cc. per 100 g.)	0.9500	Quartz sand with 0.03N KCl (2 cc. per 100 g.)	0.6292
Quartz sand with 0.001N KCl (2 cc. per 100 g.)	0.9829		
Quartzs and with 0.01N KCl (2 cc. per 100 g.)	0.8117		

The dependence of the value of n on the electrical conductivity is quite clearly expressed. The greater the latter the less the calculated value of n. A certain inconsistency at the start may be due to experimental errors. The relationship shows that as the electrical conductivity is increased the weight of the quartz sand plays an ever smaller part; *i.e.*, a lesser part is played by the quantity of dielectric, and other factors become more important. B. P. Alexandrov and G. P. Mikhailov [5] consider that the capacity of a condenser filled with a powdery dielectric is determined by a multiplicity of different capacities of the microcon-

[5] J. Tech. Phys. (Russia) 8 (No. 12): 1121 (1938).

densers which are formed within the space. Connection between these elementary condensers is established by bridges or films consisting of the aqueous solutions which are situated on the surfaces of and in the capillaries formed by the particles. Owing to their conductivity these solutions form, as it were, condenser plates separated by the particles of the substance from which the dielectric is formed. This conception may be particularly applicable to such powdery substances as quartz sand in which the water is situated on the surfaces of the particles. By varying the concentration of the solution added we alter the conditions of formation of the microcondensers, increasing them as the electrically conducting part is increased in comparison with the nonconducting. It is quite possible that this effect is the cause of the reduction in the part played by the weight, which in turn reduces the absolute value of n, since the volume of the actual dielectric, as it were, decreases during this process.

The structure and properties of such powdery substances as ground biscuit are very different from those of quartz sand. First of all they are able to retain water not only on the surface but are also able to absorb it within themselves owing to the large number of capillaries and to the colloidal properties of the substance. It is therefore to be expected that the relationships which are applicable because of a change in the electrical conductivity of biscuit will in this case be somewhat different from those in the case of quartz sand, and will undoubtedly be of greater or less influence.

We carried out the following experiments in order to make sure that electrical conductivity plays a part in measurements of the dielectric constant. First of all we prepared specimens of biscuit according to a recipe for one of the known types of sugar biscuits.[6] One specimen was prepared according to the usual recipe, the second was prepared without any addition of salt, and the third with five times as much of it as in the normal recipe. Each specimen was ground and divided into several portions of which each was then moistened to a different degree by placing over water in a desiccator.

There were thus obtained three series of samples with different moisture contents within each series. The dielectric properties of the specimens were measured in the usual manner, all the data being referred to 100 g. by means of the formula with $n = 0.7$. The results of the experiments expressed in the form of curves are given in Figure 7. The moisture contents of the specimens are laid out on the horizontal axis and on the vertical one the dielectric constant referred to 100 g. Not all the curves lie at the same level. Curve III for biscuit with a large

[6] Flour, 320 g.; sugar powder, 140 g.; invert sugar, 15 g.; maize starch, 24 g.; margarine, 60 g.; salt, 2 g.; soda, 2.2 g.; ammonia, 0.2 g.; essence, 1 g.; milk, 36 g.; melange, 24 g.

quantity of salt lies considerably lower than the others. The influence of the great salinity is quite clearly expressed.

Curves I and II lie almost on the same level. The divergence between their upper parts is very likely incorrect and may be explained by fortuitious experimental circumstances. In our opinion this coincidence is due to the small difference between the compositions which differ by only 2 g. of salt per 100 g. of sample. The sample without salt should be more correctly designated as a specimen *without the addition of refined salt,* since other salts are present in fair quantity. The addition

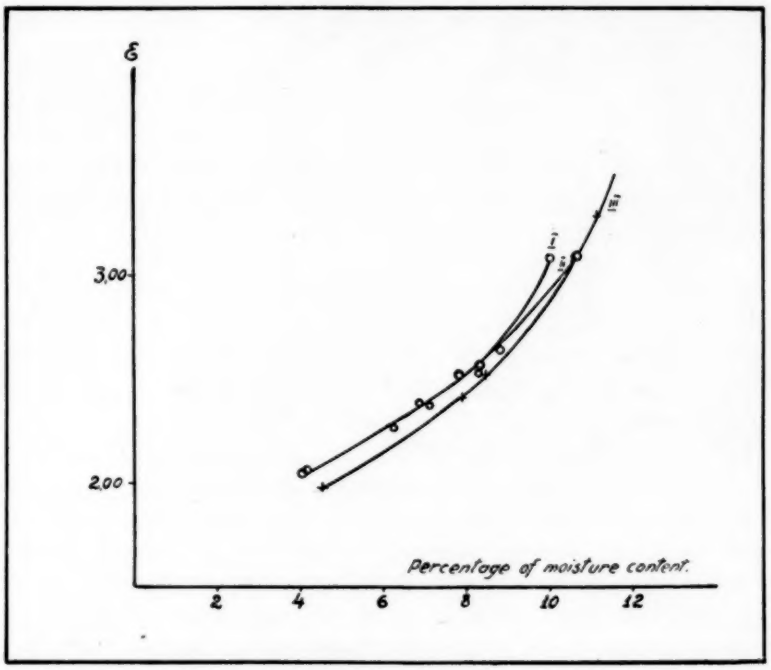

Fig. 7. Curves for biscuit with varied salt content.

of a five-fold quantity of salt causes an acute change in the ash content of the biscuit, which has its influence on the results of the measurements.

The following two conclusions may be drawn from these experiments: (1) The value of the constant n for which the best results for leveling out the differences in the packing density are obtained may be different for different substances. Preliminary experiments are necessary in each individual case. (2) The small difference in the ash content (salinity) of the biscuit does not play an appreciable part.

Insofar as concerns the influence on the value of n of such other

factors as the structure and size of the particles of powder, the size and shape of the condenser and so on, it may be supposed that they also have a certain influence. This question requires further research.

Work with Granulated Sugar

Preliminary experiments carried out in the previous work demonstrated that the dielectric constant of sugar depends upon its moisture content, but it was not finally apparent which of the formulas was most suitable for taking into account the packing density of the granulated sugar in the condenser. The results obtained with Lichtenecker's formula and our own were almost identical.

Lichtenecker's formula is

$$\log E \text{ substance} = \frac{\log E \text{ mixture}}{\delta},$$

where E mixture is the dielectric constant of a mixture of the substance with air and δ is that fraction of the volume which is occupied by the substance. This formula corresponds to our formula:

$$\log E_1 = \frac{a_1^n \log E_2}{a_2^n}, \qquad \text{when } n = 1.$$

Recalculation when the index is equal to 1 is much simpler than for n greater or less than one, and it would therefore be convenient to apply such a formula.

In order to show the applicability of one expression or another for our purpose we carried out the following experiments. First we prepared specimens of granulated sugar with crystals of different sizes. For this purpose a certain quantity of commercial granulated sugar was divided into a number of fractions according to the size of the crystals, by passing it through metal sieves of different gauges. We thus obtained three samples of fine, medium, and coarse crystals. Finally, in order to obtain still finer crystals the sugar was ground to a powder with a mortar and pestle. The measuring condenser was filled up to the mark with these substances. It was then enclosed into the instrument and finally weighed.

The measured values of the dielectric constant were referred by the formula to the standard weight of 180 g. and according to the way in which the results agreed for different specimens we judged the advantage of one or the other formula. The data are given in Table VI.

Reference to the data for 180 g. shows that the use of the formula with $n = 1$ gave very good results. Repeated determinations carried out on one and the same specimen but with different packing densities coincide better than when $n = 2/3$.

TABLE VI

MEASUREMENTS ON SUGAR PARTICLES OF VARYING SIZE

Substance	Moisture content	Diel. const. from instrument	Weight of substance	Diel. const. of sugar at 180 g. calculated with $n = \frac{2}{3}$	Mean	Diel. const. gran. sugar at 180 g. calculated with $n = 1$	Mean
	%	g.	g.				
Granulated sugar	0.01	2.169	221.0	1.964	1.962	1.873	1.882
		2.131	216.5	1.952		1.876	
		2.142	216.6	1.960		1.884	
		2.178	219.3	1.978		1.894	
		2.131	215.9	1.955		1.881	
Coarse grained fraction	0.01	2.035	200.0	1.940	1.926	1.896	1.882
		2.021	200.6	1.925		1.880	
		2.033	200.9	1.933		1.888	
		2.024	203.3	1.916		1.867	
		1.997	197.3	1.917		1.880	
Fine grained fraction	0.04	2.146	214.0	1.975	1.982	1.901	1.903
		2.207	217.5	2.011		1.926	
		2.166	216.5	1.981		1.902	
		2.149	215.3	1.972		1.897	
		2.164	218.0	1.972		1.891	
Sugar powder	0.02	2.144	213.0	1.977	1.961	1.905	1.887
		2.162	219.0	1.967		1.885	
		2.133	216.0	1.956		1.880	
		2.129	215.8	1.954		1.878	
		2.093	209.4	1.950		1.887	

The advantages of the second formula are still more obvious on comparing the data for specimens of different grain sizes. The concordance of values for the dielectric constant is very good in spite of the great difference between the weights of the substance required to fill the measuring condenser. Hence we may draw the conclusion that it is most advantageous to use the formula with the value of $n = 1$, in order to take into account the packing density of granulated sugar.

It is obvious that neither the electrical properties nor the ability of individual particles to be packed into the condenser are the same for biscuit and sugar. The results of the experiments with granulated sugar consequently confirm the conclusions of the previous section of this work.

Work with Flour

The work was carried out with specimens of 35% and 85% wheat flour. The experiments showed that the design of measuring condenser used for biscuits is not suitable for accurate measurements of the di-

electric constant of flour. The flour was not distributed uniformly throughout the condenser and formed into layers of greater or lesser density, sometimes even leaving spaces, which considerably reduced the reproducibility of results. The experiments should be continued with a condenser in which the distance between the plates is so great as not to hinder uniform distribution of the flour throughout the entire volume of the condenser.

Summary

The work was mainly devoted to verification of the applicability of dielectric measurements for the determination of the moisture content of ground biscuit and led to satisfactory results and to further methodological development of this procedure.

The question of the conditions for determination of the curve of the dielectric constant of the substance as a function of the moisture content was studied in detail.

A system of simplified calculations for practical measurements and for drawing up the curve by means of graphs and nomograms was worked out.

A method of transition from one condenser to another without lengthy calibration was developed and verified experimentally.

The question of the influence of the temperature on the results of the measurements was investigated.

A preliminary investigation was made of the question of the influence of the composition of the dielectric on the constant n in equation No. 1, used for recalculation.

A comparison was made between the applicability of formula No. 1 when $n = 2/3$ and when $n = 1$ for granulated sugar.

DOUGH IMPROVEMENT STUDIES. IV. EFFECT OF SOME FACTORS INVOLVED IN RESPIRATION ON THE BAKING QUALITY OF WHEAT FLOUR

EDWIN ZIEGLER

Buhler Bros., Uzwil, Switzerland

(Received for publication April 6, 1940)

Since Pasteur's work it has been known that a relationship exists between fermentation and oxidation. The common substrate in both cases is a triose according to Szent-Györgyi (1937, 1938, 1938a). When oxygen is lacking fermentation takes place, two hydrogen atoms of the triose being transferred to pyruvic acid, which becomes lactic acid (*e.g.* in muscle). On the other hand in the presence of oxygen we have oxidation, of which respiration—the most powerful function in cell life—is one form.

Here the triose gives off two hydrogen atoms to oxalacetic acid, which Szent-Györgyi considers the most important hydrogen carrier in respiration. This acid is converted into malic acid. Both malic and oxalacetic acid are activated by the enzyme malicodehydrase, whereas succinic acid is activated by succinodehydrase. Fumarase, a powerful enzyme contained in all tissues, makes fumaric acid take up one molecule of water and become malic acid. Paradoxically, it also makes malic acid give off water. If one of these acids is added, in the presence of fumarase, an equilibrium (fumaric : malic = 1 : 3) is reached. Succinic acid is oxidized by cytochrome, while malic acid is oxidized to oxalacetic acid. Szent-Györgyi's scheme of H transport is:

Substrate (triose) → oxalacetic acid → [malic acid ⇋ fumaric acid]
→ succinic acid → WKS—O_2

By "WKS" is meant the Warburg-Keilin system, *i.e.*, cytochrome and cytochrome oxidase.

Figure 1 shows the interrelationship of several acids involved in respiration and fermentation. Upward directions represent oxidations; those downward show reductions. Among substances which greatly increase respiration are fumaric and succinic acids (Szent-Györgyi, 1937) and adrenaline (Haurowitz, 1938). Substances known to decrease respiration are maleic acid (Thunberg), malonic acid (Quastel), and pyrophosphate (Dixon). The two last-mentioned compounds poison succinodehydrase. Cysteine is an inhibitor of yeast respiration.

Oppenheimer and Stern (1939) in their recent review of biological

oxidation, are still unable to assign a place to glutathione in cell respiration. The respiration of yeast or of animal tissues is affected neither by reduced (GSH) nor by oxidized glutathione (GSSG). Kendall and Nord suspect an intermediary form between GSH and GSSG. The hydrogenation by a donator and the dehydrogenation by an acceptor

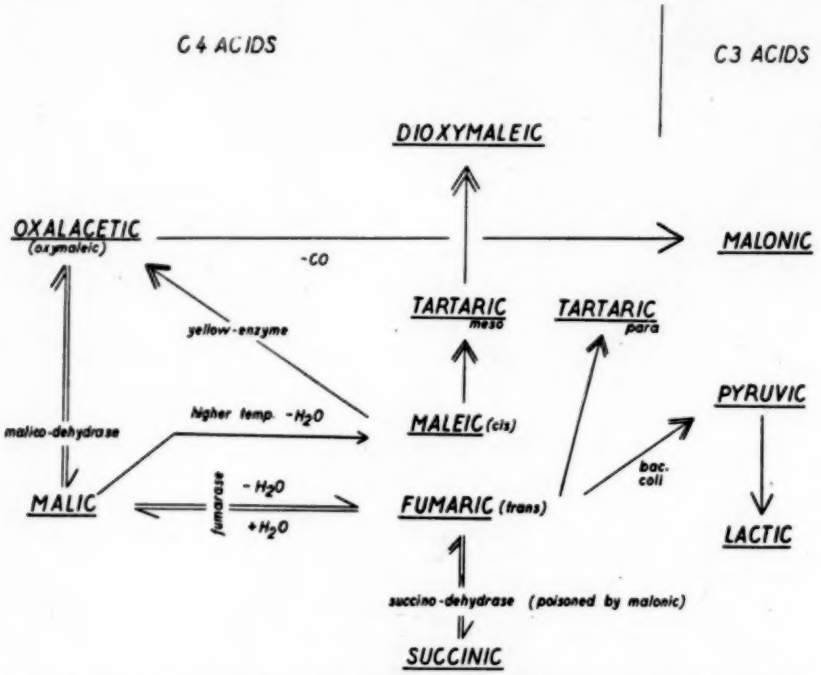

Fig. 1. Interrelationship of some acids with three and four carbon atoms involved in respiration and fermentation.

seem to be more complicated here than in the case of other carriers. Oxidation processes of interest in studying flour quality might be summarized as follows:

Oxidation process	Main substrate in wheat flour affected	Effect in relation to baking as result of oxidation of substrate
Fermentation	Carbohydrates	Production of gas (CO_2)
Respiration	Carbohydrates	Unknown
Aging	(*a*) Proteins, glutathione	Improvement of gluten quality
	(*b*) Unsaturated fatty acids	Damage to gluten quality
	(*c*) Fats (development of rancidity)	Damage to keeping quality

Freilich and Frey (1939) have demonstrated that mixing in oxygen will partly eliminate the bad effect of germ, cysteine, and glutathione added to a dough. The object of the present investigation was to

test whether the action of some factors involved in or influencing respiration can help to dehydrogenate (*e.g.*, glutathione) or to activate oxygen so that a higher degree of oxidation of the dough results, than that found by the authors mentioned. The tests might also help to explain the mode of action of oxidation in dough, known to lead to the improvement of bread.

Materials

Papain has recently been isolated in a pure state by Balls and Lineweaver (1939). Bergmann, Fruton, and Fraenkel (1937) believe that papain contains two enzymes, which they call papain I and papain II. It has been shown by Rupp and Bailey (1937) that small increments of papain did not cause any degradation in a dough.

Papain or papayotin is activated by glutathione due to the reduction of the enzyme itself (Bersin, 1933). Purr (1935) found that the enzyme is inactivated by alloxan. Papain is slowly destroyed in a pure state. It is not affected by aeration alone but aeration in the presence of copper and iron ions caused partial inactivation (Hellermann and Perkins, 1934).

Commercial preparations of papain contain sulphur in the form of cystine, methionine, and some unidentified compound (Schöberl and Fischer, 1939). At the same time several "impurities" are reported in commercial preparations: a natural papain activator, sterols, a lipase, a reducing dyestuff, carbohydrates, and zinc, the latter an enzyme poison (Schöberl and Fischer, 1939; Ganapathy and Sastri, 1938; Maschmann and Helmert, 1934; Sandberg and Brand, 1925).

For our work we used Merck's papain 1 : 20. The adrenaline used was a Merck product marked Ph. helv. V., which is the *l*-adrenaline base. The aneurin (thiamin) was also from the same firm. The malic acid was the inactive compound. The reduced glutathione and ascorbic acid were from Hoffmann-LaRoche in Basle (Switzerland).

The gluten flour came from a starch factory. It had retained its swelling power and contained 3.6% starch, 0.69% fat, and 8.2% moisture.

Methods

Titrations were carried out with 0.0020*N* iodine to determine the degree of oxidation of glutathione, as previously described (Ziegler, 1940, 1940a). It takes 1.63 cc. *n*/500 iodine to completely oxidize 1.00 mg. of reduced glutathione. The completely oxidized glutathione still needed 0.15 cc. of iodine. This method was shown to differentiate between the activity of bromate and the inactivity of chlorate as oxidizing agents of reduced glutathione.

Turbidity measurements were made by a method which is a com-

bination of the Berliner (1929, 1937) gluten swelling determination and of the method used by Flohil (1936), which enabled us to determine rapidly the activation or inhibition of papain action on gluten in the presence or absence of glutathione. Flohil set away washed-out gluten (divided into small pieces) with a trace of boric acid for 48 hours at 30°C. According to the substance added the supernatant liquid was more or less turbid after the two days. To shorten the test we applied the Berliner method of allowing the gluten to swell and dissolve in *n*/50 lactic acid at 27° for 60 minutes in a rotating thermostat. The turbidity was measured with a photoelectric cell after centrifuging. Objections have been raised against the Berliner method of determining gluten quality, mainly by Bungenberg de Jong (1939) on the grounds that it only considers one of the components. As mentioned by Berliner (1931), gliadin goes into solution from all glutens during the test, but in the case of glutens classified as weak, a breakdown occurs at the same time, whereas strong glutens do not disintegrate. Whatever the exact value of the Berliner method may be—we have always found it useful in classifying small samples of wheat—it proved very satisfactory for the present tests, in spite of the drawback of having to work in a strongly acid medium (pH 3). Four different photometers, designated as B_1, B_2, L, and DC, were used so that all figures here reported cannot be directly compared. Not every photometer with a photoelectric cell can be used for these measurements because of the

TABLE I

TITRATION OF MIXTURES OF GLUTATHIONE AND DIFFERENT AGENTS

(In each case 1 mg. reduced glutathione in 10 cc. distilled water. Reaction time 1 hour.)

No.	Addition		*n*/500 iodine
		mg.	*cc.*
1	None	—	1.70
2	Potassium bromate	50	0.15
3	Ammonium metavanadate	5	1.40
4	Ammonium metavanadate	20	2.70 [1]
5	Fumaric acid	5	2.00
6	Fumaric acid	20	2.00
7	Succinic acid	5	1.75
8	Maleic acid	50	1.90
9	Malonic acid	5	2.30 [1]
10	Nicotinic acid amide	5	1.65

[1] Blue color disappears.

Tyndall effect; small concentrations of suspended particules throw a greater amount of light on the cell than would be the case when pure water is placed between the light source and the cell. The photometer

can be built so that the smallest concentration of suspended particles corresponds to an increase in light absorption, as measured by the microammeter. That was the case with our measurements.

When gluten flour was used alone, only a slight turbidity was observed (*i.e.* low percentage of absorption). On adding a fair amount of protease a high absorption is found (Table II, 1–5). Figure 2

TABLE II

EFFECT OF DIFFERENT ADDITIONS ON THE TURBIDITY OF GLUTEN FLOUR IN ACID SOLUTION

(0.5 g. gluten flour in 20 cc. *n*/50 lactic acid, shaken for 1 hour at 27°C., measurement in an electric photometer after centrifuging.)

No.	Papain	Addition		% light absorbed	Inhibition partial (*) or total (**)
	mg.	*mg.*		*Photometer B1*	
1	—	—		15	
2	5	—		17	
3	10	—		32	
4	20	—		73	
5	30	—		83	
6	—	7.5	Glutathione (reduced)	16	
7	30	—		83	
8	30	5	Potassium bromate	14	**
9	30	5	Potassium iodate	12	**
10	30	5	Potassium chlorate	83	
11	30	30	Ascorbic acid	82	
12	30	30	Dehydroascorbic acid	42	*
				Photometer B2	
13	20	—		92	
14	20	5	Potassium bromate	15	**
15	20	5	Succinic acid	93	
16	20	5	Fumaric acid	86	
17	20	5	Malic acid	88	
18	20	5	Maleic acid	43	*
19	20	5	Malonic acid	92	
20	20	5	Adrenaline	87	
21	5	—		24	
22	5	5	Glutathione (reduced)	87	
23	5	5 5	Glutathione (reduced) Potassium bromate	14	**
24	5	5 5	Glutathione (reduced) Maleic acid	30	*

shows the rise in light absorption with increasing amounts of papain for two photometers with somewhat different characteristics. As the white colloidal solution always remains translucent, there is always a certain amount of light which falls on the cell. This explains why the absorption measured never reached 100%, *i.e.*, complete darkness. Figure 3 shows the effect of activation of papain by increasing amounts of glutathione. Five mg. and 7 mg. of GSH gave 91% and 91.5%. Figure 4 was obtained by progressive dilution with *n*/50 lactic acid of

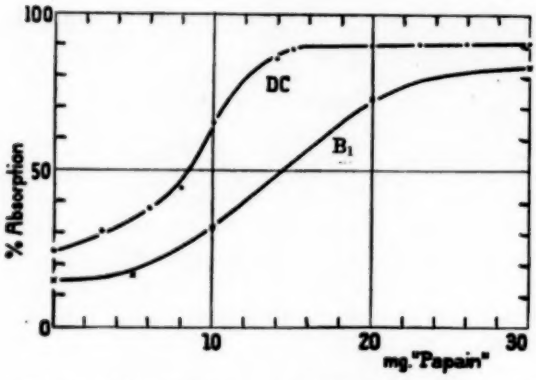

Fig. 2. Turbidity measurements with photometers DC and B_1. Different amounts of "papain" added to 0.5 g. of gluten flour. Distilled water taken as 0% absorption.

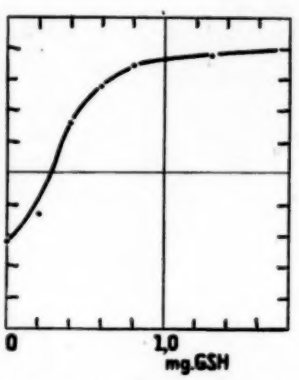

Fig. 3. Turbidity measurements with photometer DC. Different amounts of GSH added to 0.5 g. of gluten flour + 5 mg. papain.

one of the solutions used for Figure 3. The papain preparation did not contain any heat-stable activator, as even a large amount of papain after boiling for 30 minutes did not increase absorption (Table III, 34–37). In fact a slight decrease (Nos. 36 and 37) was measured which cannot yet be interpreted. Twenty mg. of boiled papain had no effect on 15 mg. of unboiled papain.

Figures 2, 3, and 4 show that, with photometer DC, in the presence

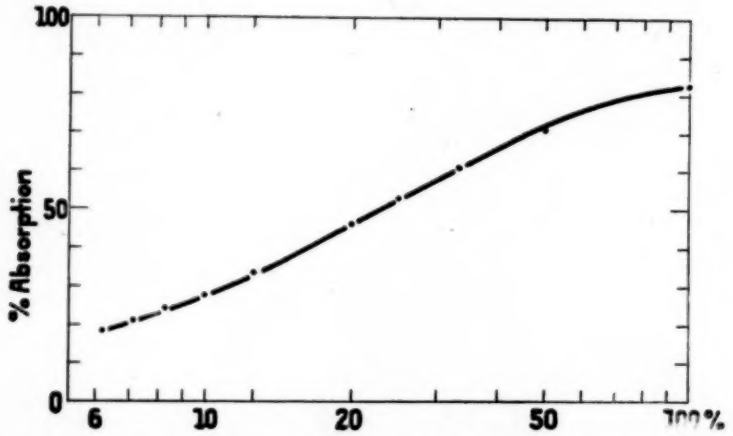

Fig. 4. The dilution of a solution from a turbidity test containing gluten flour, papain, and glutathione as a logarithmic function of absorption.

of not more than 15 mg. of papain, even the smallest increment of an inactivator could be measured. The same applies to the amount of glutathione in the presence of 5 mg. of papain. Here (Fig. 3) 1.5 mg. of GSH would be enough. We used 5 mg. of GSH throughout, be-

TABLE III

Effect of Different Additions on the Turbidity of Gluten Flour in Acid Solution

(0.5 g. gluten flour in 20 cc. *n*/50 lactic acid, shaken for 1 hour at 27°C. Turbidity measured with an electric photometer after centrifuging.)

No.	Papain	GSH	Addition		% light absorbed	Inhibition partial (*) or total (**)
	mg.	*mg.*	*mg.*		*Photometer L*	
1	–	–	—		31	
2	5	–	—		42	
3	5	5	—		94	
4	5	5	5	Potassium bromate	29	**
5	5	5	5	Potassium iodate	32	**
6	5	5	5	Potassium chlorate	93	
7	5	5	5	Potassium chlorate+osmic acid[1]	62	*
8	5	5	5	Ammonium persulphate	37	**
9	5	5	5	Ammonium metavanadate	28	**
10	20	–	5	Ammonium metavanadate	19	**
11	5	5	5	Alloxan[2]	72	*
12	5	5	5	Succinic acid	95	
13	5	5	20	Succinic acid	90	
14	5	5	5	Fumaric acid	95	
15	5	5	20	Fumaric acid	90	
16	5	5	5	Malic acid	96	
17	5	5	20	Malic acid	90	
18	5	5	5	Malonic acid	94	
19	5	5	20	Malonic acid	89	
					Photometer DC	
20	–	–	—		24	
21	5	–	—		36	
22	5	5	—		94	
23	5	5	5	Adrenaline (oxidized by heating)	79	*
24	5	5	5	Adrenaline (red)	94	
25	5	5	5 5	Adrenaline (red) Copper sulphate	48	*
26	5	5	5	Copper sulphate	63	*
27	5	5	5	Aneurin (thiamin)	95	
28	5	5	5	Nicotinic acid amide	91	
29	5	5	5	*l*-asparagine	91	
30	5	–	5	*l*-asparagine	30	
31	5	5	5	"Chloramin" (Heyden)	90	
32	5	5	20	"Chloramin"	45	*
33	20	–	5	"Chloramin"	25	**
34	–	–	—		24	
35	–	–	20	Boiled papain	23	
36	5	–	—		30	
37	5	–	20	Boiled papain	26.5	
38	–	5	—		24	

[1] One drop 2% osmic acid.
[2] Not all dissolved.

cause we had carried out the preliminary tests in that way, but this implies that the oxidizing agents would first have to inactivate the surplus GSH before any oxidation of the glutathione could be detected by the photometer.

The tests and measurements were carried out in test tubes which, filled with water, differed to the extent of 0%–1% in absorption. The usual motor-driven centrifuge could not be used, so that we had to resort to a hand-driven centrifuge which could hold the test tubes. The irregular centrifuging accounts for most of the differences found when tests were duplicated.

Glutathione (GSH) alone (*i.e.*, in the absence of papain) had no effect on turbidity (Table II, 1, 6; Table III, 34, 38), from which we deduce that the gluten flour contained no active protease and was not attacked by GSH under these conditions. A similar test, but with 0.5 g. of fresh wet gluten alone and in the presence of 5 mg. of GSH, gave 22% and 26%. In the wet gluten no amount of active protease of any importance seemed to be present. A mixture of papain and glutathione was inactivated by the addition of bromate, iodate, persulphate, and metavanadate, whereas chlorate was without effect (Table III, 4–10). It has been shown (Ziegler, 1940a) that, by the titration method referred to above, chlorate in the presence of osmic acid will oxidize glutathione, an effect also revealed by the turbidity method (Table III, 6, 7). Metavanadate was included because its action has been studied in connection with protease (Read and Haas, 1938; Hale, 1939).

The baking tests were carried out with 200 g. of flour from a 1 : 1 mixture of Canadian and Argentine wheat, which could be improved by bromate. After mixing, the dough was divided into two parts. The dough contained 2% salt, 3% yeast, 0.5% maltose, but no fat. The mixing was done in a hand-driven iron mixer so as to avoid strong mechanical treatment and possible traces of copper, shown recently by Bungenberg de Jong and Klaar (1939) to be of importance when dough is mixed in oxygen. Even without oxygen a copper bowl can cause a reduction in loaf volume, as observed by Frank (1932). In all cases a current of oxygen was fed into the mixer, because the problem was to see whether respiratory carriers could be used to transport oxygen for the oxidation of dough.

Factors Involved in Animal Respiration

Three important factors in respiration are not included in this study, namely cytochrome, oxalacetic acid, and hemoglobin. The four substances mentioned below are all either easily reduced or oxidized. Bungenberg de Jong (1939) explained that in a dough it does

not seem to matter whether the reduced or oxidized form of such redox substances is added.[1]

Succinic acid ($COOH.CH_2.CH_2.COOH$).—No substance is oxidized by tissues as rapidly as succinic acid. If oxygen is present, succinic is oxidized to fumaric acid, but this reaction can be poisoned by malonic acid and by pyrophosphate (Szent-Györgyi, 1937). Succinic acid reduced cytochrome *c* much faster than any other substrate (Straub, 1937). The acid is activated by succinodehydrase (which will be referred to under malonic acid) and increases respiration. If succinate is added to tissue, more oxygen will be taken up than the tissue ever consumes in normal respiration (Szent-Györgyi, 1937). In plant respiration polyphenols take the place of this acid (Szent-Györgyi, 1938a).

Kent-Jones (1934) mentioned succinic acid as a useful and powerful improver. Alcoholic fermentation transforms about 0.01%–0.02% of a flour into succinic acid; the higher the percentage of yeast, the more succinic acid formed (Guillemet, 1937). The presence of ammonium chloride prevents this formation (Hoffman, 1917). Kent-Jones (1939) believes that the improving action of some substances may be due to the formation of abnormally large quantities of succinic acid during fermentation. There is however no direct evidence of this yet.

Fumaric acid ($COOH.CH:CH.COOH$).—This is the isomer of maleic acid, the latter being the cis-form, whereas fumaric acid is the trans-form (Fig. 1). Krebs (1938) states that fumarate and molecular O_2 are equally good as H-acceptors if glucose is the donor. In this case glucose is oxidized and fumarate reduced to succinic acid. Adrenaline acts similarly on glucose (see further on). Krebs assumes that the total H of glucose passes through fumaric acid when glucose is dehydrogenated. Fumaric acid increases respiration (Szent-Györgyi, 1937).

Malic acid ($COOH.CHOH.CH_2.COOH$).—This acid yields oxalacetic acid and succinic acid on oxidation and reduction respectively (Fig. 1). Malic acid is a very important link in the chain of reactions in respiration. It should not be confused with maleic or malonic acids. In nature *l*-malic acid is found, and its presence in wheat has been reported (Nelson and Hasselbring, 1931). Grewe and Child (1930) used the acid in place of tartrate in cake.

Alloxan.—Szent-Györgyi (1938a) believes that flavines may possibly be placed between malic and fumaric acid in the scheme of respiration. The main component of flavines is alloxazine, which combines with a sugar radicle to form lactoflavine (riboflavin, vitamin B_2), a widely distributed substance in nature. An important part of the

[1] Sullivan *et al.* (Cereal Chem. **17**: 507–528, 1940) make a similar statement.

alloxazine molecule is alloxan, and H-acceptor, which can be reduced to dialuric acid.

Since alloxan inactivates papain (Purr, 1935), oxidizing the SH group (Dixon, 1939), it should prove useful as a bread improver. It has already been used as such, but fairly large quantities are necessary (Bungenberg de Jong, 1939; Jørgensen, 1938). According to the latter, dialuric acid—the reduced form—proved even better than alloxan According to Belgian patent 415,652 (1936) 0.002% is the usual amount necessary for improving a dough.

The results obtained with these four products were as shown in the tables. Table I shows that succinic acid and its oxidation product, fumaric acid, did not oxidize glutathione directly.

In the baking test (Table IV) alloxan, succinic, and fumaric acids showed an improvement, whereas malic acid did not. Only in the first case does the effect seem to be due to the inhibition of papain action (Table III, 11), although this was only partial, because the alloxan was not completely dissolved. Neither of the three organic acids inhibited the action of the papain preparation alone nor in the presence of glutathione (Table II, 15–17; Table III, 12–17).

Farinograph tests were made using 85% flour, 15% commercial wheat germ, and 0.1% succinic, fumaric, or malonic acids, omitting malic acid. After mixing and an hour's rest, the unchanged strong i ncrease in mobility showed that none of the acids had any beneficia effect whatever, even in the presence of flour constituents. Even if the germ did contain succinodehydrase, the poisoning of this enzyme with malonic acid did not improve the physical condition of the dough.

Factors Influencing Respiration

Numerous substances are known to influence respiration in some way or other. Four of these were tested.

Adrenaline (*epinephrine*).—This is the first hormone to be synthesized. It exists in blood in a concentration of 1 in 1 billion, and is also found in the suprarenal glands. Adrenaline has been classified among the oxytrope catalysts; *i.e.*, it needs no intermediary catalysts to take up oxygen. Adrenaline is an unstable weak base, a strong poison, and a strong reducing agent. It is readily oxidized by oxygen, yielding a brown-colored substance; a ketone named adrenalone is produced (Sherman, 1928). Adrenochrome, another oxidation product, has also been obtained from adrenaline. Sherman also states that the rate of oxidation of glucose is increased by adrenaline. It acts quickly but only for a short time, whereas thyroxin acts more slowly but for a longer time. Because of this fact, thyroxin might be found to give

better results as a dough improver than adrenaline, although both are out of the question for practical use.[2]

Adrenaline is sparingly soluble in cold water. In warm water it oxidizes and is then fairly soluble. It can be dissolved in water at 60°C. If heating is short the solution is red; on prolonged heating it becomes yellow. Neither of these solutions improved loaf volume alone (Table IV, 8, 9). In the presence of a trace of copper a better oxidation of the dough was obtained, and in one test even a badly overtreated loaf was produced. The oven spring was good.

These results can be explained by the action on glutathione, as shown by results in Table III (23–26). Here too it was found that a mixture of oxidized adrenaline with a trace of copper was better than each separately. Copper sulfate alone also produced a partial oxidation. The importance of copper in dough oxidation has been emphasized by Bungenberg de Jong and Klaar (1939) and by Berliner (1939).

Ascorbic acid ($C_6H_8O_6$).—This acid (vitamin C) plays an important part in Szent-Györgyi's (1938a) system of oxidation of plant tissues, together with succinic acid and quinone. The oxidation of ascorbic acid leads to dehydroascorbic acid. Bezssonoff and Woloszyn (1938) found a form intermediate between the two. On addition of iodine at pH below 4 the first oxidation stage is formed, at high pH the dehydro form occurs (Wurmser, 1935). In tissues rapid reduction of the dehydro acid is brought about by glutathione (Borsook *et al.*, 1937; Kohman and Sanborn, 1937). Since its discovery as a bread improver by Jørgensen (1935) several authors have reported favorably on its efficiency as such.

Alpha-amylase was shown by Rupp and Bailey (1937) to alter the physical properties of gluten. Part of the beneficial action of dehydroascorbic acid in dough may be due to the fact that this acid inhibits alpha-amylase action (Euler *et al.*, 1934; Hanes, 1935; Weidenhagen, 1936). Melville and Shattock (1938) demonstrated that the dehydro acid is a more efficient improver than ascorbic acid itself and that the improving action is due entirely to the oxidation product. They also found that there exists in flour a mechanism whereby this oxidation takes place. The oxidation of ascorbic acid can, however, also be rapidly brought about by a peroxidase system (Fischer, 1937). Pulkki and Puutula (1938) determined the ascorbic acid content of germinating wheat.

Hopkins and Morgan (1936) stated that in the absence of ascorbic acid oxidase, dehydroascorbic acid is reduced five times slower than in its presence. By titration Ziegler (1940) found that dehydroascorbic

[2] Adrenaline strongly inhibits the autoxidation of unsaturated fatty acids (Oppenheimer and Stern, 1939, p. 121).

acid alone oxidized glutathione only about half as rapidly as bromate did. Melville and Shattock (1938) state that the action of dehydroascorbic acid, on a weight-for-weight basis, was equal to bromate as an improver. This difference may be explained by the presence of the oxidase in flour. We have, however, always found dehydroascorbic acid the weaker of the two in its action. The titration curves (Fig. 5) show that, in the absence of flour constituents and especially of ascorbic acid oxidase, dehydroascorbic acid oxidizes glutathione slower than the same amount of bromate does; *i.e.*, it is reduced much slower than bromate is. The action is shown clearly in the turbidity tests (Table II, 11, 12). Ascorbic acid, being a reducing agent, could not be expected to inhibit papain action in the absence of an oxidase. Even 30 mg. of dehydroascorbic acid did not inhibit as strongly as did 5 mg. of bromate.

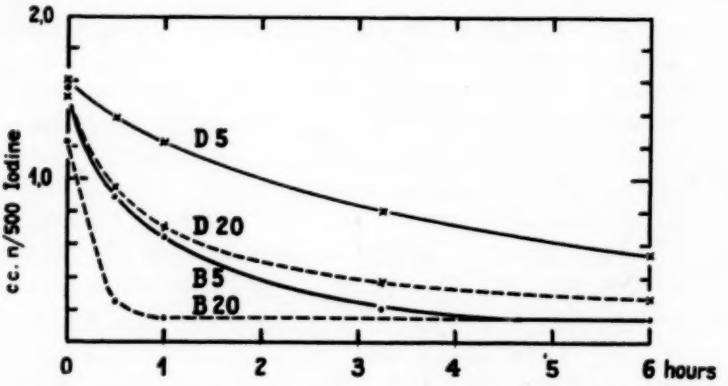

Fig. 5. Rate of oxidation of glutathione by potassium bromate (B) and dehydroascrobic acid (D). In all cases 1 mg. of GSH in 10 cc. of distilled water was treated at room temperature with 5 mg. and 20 mg. of the oxidizing agents.

Maleic acid.—This compound and fumaric acid are isomers (Fig. 1). Maleic is the stronger acid. It does not enter into respiration itself; in fact it was shown by Thunberg to be a strong poison of respiration (Szent-Györgyi, 1938), interacting with succinodehydrogenase and thus stopping any further O_2 uptake (Morgan and Friedmann, 1938a). The latter (1938, 1938a) found that maleic acid can combine with glutathione to form a thiosuccinic acid derivative inhibiting the catalyzing action on enzymes by the SH compound. They showed that the interaction does not go on to completion. Maleic acid is stated by Triebold *et al.* (1933) to be a useful agent against rancidity, *i.e.*, against a certain type of oxidation of fat.

As previously reported (Ziegler, 1940) and again shown in Table I (8), maleic acid did not oxidize glutathione within an hour. Added

to a dough it had no good effect on oven spring and a decidedly deleterious effect on crumb texture.[3] The turbidity test, however, showed a marked inhibition by maleic acid, of the glutathione activation of papain action, although the effect is not quite so strong as with bromate (Table II, 21–24). The acid also had an inhibiting effect on the papain preparation itself (Table II, 18), in agreement with Morgan and Friedmann (1938a).

Malonic acid ($COOH.CH_2.COOH$).—Minute quantities of malonic acid poisoned respiration almost as cyanide does (Szent-Györgyi, 1937). This is because the oxidation of succinic to fumaric acid is inhibited (Fig. 1). Either pyrophosphate or malonic acid specifically poisons the enzyme succinodehydrase. This enzyme can reduce papain and cystine (Bersin, 1933, 1935). It occurs in all animal tissues, yeast, and bacteria, although some tissues are said to be deficient (Oppenheimer and Stern, 1939). By the use of malonic acid it might then be possible to inhibit the reduction of papain in dough, *i.e.*, to make it inactive, which should result in improved baking quality. A Berlin firm at one time put a malonic acid product called "Matagen" on the market and Neumann (1929), without giving the actual analysis, mentions that 0.05%–0.1% Matagen had a decided improving effect in a dough. It has the advantage of being volatile, so that no foreign substance is left in the bread. Traces of this acid have been found in wheat itself (Nelson and Hasselbring, 1931).

The turbidity test (Tables II, 19; Table III, 18, 19) showed no action against the reduction of papain, in spite of the fact that in all probability in our tests no succinodehydrase, which would tend to reduce papain, was present. The effect claimed for malonic acid does not seem to be explained by an action on glutathione. Our baking tests did not reveal malonic acid as a very good improver (Table IV, 7). As already mentioned, the farinogram of a flour and germ dough was not improved by malonic acid addition.

Other Factors

Aneurin (*thiamin, vitamin* B_1).—This strongly alkaline, sulphur-containing vitamin is involved in the conversion of pyruvic acid to acetaldehyde and CO_2 (Williams, 1939), and is therefore important in alcoholic fermentation (Schultz, Atkin, and Frey, 1937). We are interested in aneurin as a redox substance; on oxidation thiochrome is formed, on reduction a dehydro compound (Lippman, 1936; Boulanger, 1938). The latter can again be oxidized to aneurin. Recently Schultz, Atkin, and Frey (1939a) have determined the vitamin B_1 content of wheat, flour, and bread.

[3] Further baking tests confirmed the results.

TABLE IV

EFFECT OF ORGANIC ADDITIONS + OXYGEN ON LOAF VOLUME

(1 mg. organic additions and in two cases 0.05 mg. copper or ferro salt were added per 100 g. flour from a Canadian-Argentine wheat mixture. Doughs mixed in hand-driven iron mixer with addition of oxygen.)

No.		Volume in %	Improvement
1	Untreated	100	
2	Potassium bromate	113	*
3	Succinic acid	106	*
4	Fumaric acid	105	*
5	Malic acid(inactive form)	97	
6	Alloxan	110	*
7	Malonic acid	104	
8	Adrenaline (red)	100	
9	Adrenaline (yellow)	102	
10	Adrenaline (yellow) + $CuSO_4$[1]	110	*
11	Adrenaline (yellow) + $FeSO_4$	100	
12	Aneurin (thiamin)	100	
13	Nicotinic acid amide	100	
14	Asparagine	100	
15	"Chloramin" (Heyden)[2]	97	

[1] 0.05 mg. $CuSO_4$ alone gave 106%. [2] 2.5 mg. used.

Nicotinic acid amide ($C_5H_4N.CONH_2$).—This is the pyridine nucleus of codehydrase I (cozymase) and of codehydrase II and can be reversibly hydrogenated, taking up and giving off two H atoms, as was first shown by Warburg. The vitamin B factor held responsible for "black tongue" in dogs has been obtained in a pure state and identified with nicotinic acid amide (Elvehjem; reported by Haurowitz, 1938).

Asparagine.—This amino acid has been found to inhibit amylase action (Filipowitz, 1931) but to stimulate maltose fermentation (Schultz *et al.*, 1939), *i.e.*, just the opposite to what formaldehyde does. It occurs in the germ of many plants and was found to have a deleterious effect on baking quality (Willard and Swanson, 1913; Bull, 1937) although Sullivan *et al.* (1936) state that it has only a slight shortening effect.

"Chloramin" (p-sodium toluolsulphochloramide).—This well-known disinfectant was found to inhibit the attack of papain on gelatin by Jørgensen (1938), thus confirming the findings of Elion.

Our tests with the foregoing substances show the following results:

Aneurin and nicotinic acid amide did not inhibit glutathione-activated proteolysis (Table I, 10; Table III, 27, 28).

Asparagine was included to see whether its deleterious effect can

be brought into relation with proteolysis. Results in Table III show that it neither activated the papain (21, 30) nor did it decrease the glutathione activation (22, 29).

As was expected as a result of Elion's and Jørgensen's work, "chloramin" (Heyden) decreased the turbidity which the papain preparation would have produced with or without added glutathione, although large amounts were needed, as mentioned by Jørgensen (Table III, 31–32).

The gassing power of the nicotinic acid amide and asparagine doughs was rather weak; that of the "chloramin" dough was poor. The loaf volume (Table IV) revealed nothing noteworthy. The texture was fair in all four cases.

Discussion

The improvements in baking quality obtained with oxygen are always less than that obtained with bromate, as was also shown by Freilich and Frey (1939). It was thought that a catalyst might be found among substances known to increase or to act as carriers in respiration, so that the oxygen could improve dough to a greater extent. This could not be demonstrated.[4] The results found have been summarized in Table V.

The iodine titration method, as well as the turbidity test used, have both shown that the effect of the following flour improvers can (at least partly) be explained by their oxidation of glutathione: bromate, iodate, persulphate, and dehydroascorbic acid. Chlorate alone did not react, which is further confirmation of the validity of the results obtained by these two methods. These same substances also inhibited the breakdown of gluten by a commercial papain preparation, seemingly in the absence of an activator. The results with papain, however, cannot be interpreted as proving that the oxidizing agents act directly on the pure enzyme, as the preparation may have contained an activator, although no heat-resistant activator could be detected. So far no published data have been found on work with *pure* papain.

The turbidity test could be developed as an excellent method, especially if a real nephelometer were used. It proves useful for rapidly controlling the proteolytic action of various papain preparations, as these can be allowed to work on exactly the same substrate (gluten flour, which can be stored for several years) and under the same conditions.

Baker and Mize (1939) contend that in the absence of both yeast

[4] Weil-Malherbe (referred to by Oppenheimer and Stern, 1939) found that succinic acid, when added to animal tissues, behaves differently from that actually found in these tissues. The same held for fumaric acid and malic acid.

TABLE V

RELATIONSHIP BETWEEN LOAF VOLUME INCREASE AND INHIBITION OF PROTEOLYSIS FOR SOME ORGANIC AND INORGANIC SUBSTANCES

Oxidation of glutathione and/or inhibition of proteolytic action		
Negative		Positive
Improvement in loaf volume		
None	Slight	Definite
INORGANIC OXIDIZING AGENTS		
Chlorate		Bromate Iodate Persulphate Metaranadate
FACTORS INVOLVED IN ANIMAL RESPIRATION		
Malic acid (inactive)	Succinic acid Fumaric acid	Alloxan
FACTORS INFLUENCING RESPIRATION		
	Malonic acid	Adrenaline ($+Cu^{++}$) Dehydroascorbic acid Maleic acid[1]
FURTHER FACTORS		
Aneurin Asparagin		

[1] Poor oven spring and coarse texture.

fermentation and mechanical action, bromate has little apparent effect. The function of these two factors is probably the better distribution of the oxidizing agent in the dough, which enables it to reach the glutathione and perhaps the proteolytic enzyme more easily. Neither fermentation nor mechanical treatment is necessary for the inactivation of proteolysis itself.

Summary

The results herein reported are in line with the theory of Jørgensen (1935) which is proving a useful basis for the explanation of the mechanism of dough improvement due to the inhibiting action of oxidizing agents on proteolysis.

Factors which increase respiration (succinic and fumaric acid and adrenaline) had a certain improving action on baking quality, although only adrenaline, in the presence of a trace of copper, actually inhibited proteolysis.

Of the two factors studied which poison respiration (malonic and maleic acid), malonic had no effect on proteolysis. Maleic acid did inhibit glutathione activation even in strong acid medium. Neither class of factors that increase or poison respiration seems to have a direct effect as such on baking quality.

The rate of oxidation of glutathione by dehydroascorbic acid in the absence of oxidase was compared to that by bromate and shown to be much slower.

The turbidity method used proved useful, rapid, and simple. It can be used to determine which agents improve dough quality due to their action on glutathione.

Acknowledgment

Thanks are due Miss G. Schärer and especially Mr. F. Brassel for assistance in carrying out this investigation.

Literature Cited

Baker, J. C., and Mize, M. D.
1939 The relation of mechanical stirring to sponge doughs. Cereal Chem. **16**: 294–295.
Balls, A. K., and Lineweaver, H.
1939 Isolation and properties of crystalline papain. J. Biol. Chem. **130**: 669–686.
Bergmann, M., Fruton, J. S., and Fraenkel, H.
1937 Proteolytic enzymes. XV. J. Biol. Chem. **119**: 35–46.
Berliner, E.
1931 Einige Bemerkungen über die Kolloidchemie saurer Kleberlösungen. Mühlenlab. **1**: 13–14.
1939 Einige Schnellanalysen für das Mühlenbetriebslaboratorium. Mühlenlab. **9**: 57–64.
—— und Koopmann, J.
1929 Kolloidchemische Studien am Weizenkleber nebst Beschreibung einer neuen Kleberprüfung. Z. ges. Mühlenw. **6**: 57–63.
—— und Kranz, W.
1937 Kolorimetrische Messungen als Schnell- und Mikrobestimmungen. Mühlenlab. **7**: 89–94.
Bersin, Th.
1933 Über die Einwirkung von Oxydations- und Reduktionsmitteln auf Papain. II. Z. physiol. Chem. **222**: 177–186.
1935 Thiolverbindungen und Enzyme. Erg. der Enzymforschung. **4**: 68–97.
Bezssonoff, N., and Woloszyn, M.
1938 Sur l'existence d'une forme oxydée, intermédiaire entre la vitamine C et l'acide déhydroascorbique. Bull. Soc. chim. biol. **20**: 93–121.
Borsook, H., Davenport, H. W., Jeffreys, C. E. P., and Warner, R. C.
1937 The oxidation of ascorbic acid and its reduction in vitro and in vivo. J. Biol. Chem. **117**: 237–279.
Boulanger, P.
1938 Les récentes recherches sur la chimie des vitamines B. Bull. Soc. Chim. Biol. **20**: 516–553.
Bull, Joan
1937 An investigation into the cause of the degrading effect of wheat germ on the baking quality of flour. Cereal Chem. **14**: 244–256.
Bungenberg de Jong, H. L.
1939 Proteins in the flour industry. Pp. 160–180 in *Symposium on Proteins*, Amsterdam. Nov., 1938.
—— and Klaar, W. J.
1939 Ein Beitrag zur Kenntnis von Oxydationserscheinungen im Teig. Mühlenlab. **9**: 113–116, 123–128, 147–150.
Dixon, M.
1939 Biological oxidations and reductions. Ann. Rev. Biochem. **8**: 1–36.
Euler, H. v., Karrer, P., and Zehender.
1934 Das Verhalten von Vitamin C. Helv. Chem. Acta. **17**: 157–162.
Filipowitz, B.
1931 Influence of proteins and protein derivatives on the enzymic hydrolysis of starch by malt diastase. Biochem. J. **25**: 1874–1884.

Fischer, Marie
1937 Die Oxydation der Ascorbinsäure durch peroxydatische Systeme. Biochem. Z. **292**: 271–275.
Flohil, J. T.
1936 The effect of chemical flour improvers on proteolytic action in relation to the gas-retaining capacity of fermenting doughs. Cereal Chem. **13**: 675–689.
Frank, E. N.
1932 A note on an effect of metal in mixing bread doughs. Cereal Chem. **9**: 636–637.
Freilich, J., and Frey, C. N.
1939 Dough oxidation and mixing studies. III. Cereal Chem. **16**: 503–512.
Ganapathy, C. V., and Sastri, B. N.
1938 Natural activators of papain. Proc. Ind. Acad. Sci. **8B**: 399–404 (Chem. Ab. **33**: 2553 (1939).
Grewe, E., and Child, A. M.
1930 The effect of acid potassium tartrate as an ingredient in angel cake. Cereal Chem. **7**: 245–250.
Guillemet, M. R.
1937 Le problème du pain. Vol. 2, Actualités scientifiques et industrielles, No. 560. Hermann et Cie, Paris.
Hale, W. S.
1939 The proteinase in wheat flour. Cereal Chem. **16**: 695–702.
Hanes, C. S.
1935 Reversible inhibition of β-malt-amylase by ascorbic acid and related compounds. Biochem. J. **29**: 2588–2603.
Haurowitz, F.
1938 Fortschritte der Biochemie. Teil III. (1931–38). Verlag Steinkopff. Leipzig.
Hellermann, L., and Perkins, M. E.
1934 Activation of enzymes. II. J. Biol. Chem. **107**: 241–255.
Hoffman, C. H.
1917 Utilization of ammonium chloride by yeast. J. Ind. Eng. Chem. **9**: 148–151.
Hopkins, F. G., and Morgan, E. J.
1936 Some relations between ascorbic acid and glutathione. Biochem. J. **30**: 1446.
Jørgensen, H.
1935 Über die Natur der Einwirkung von Kalium-Bromat und analogen Stoffen auf die Backfähigkeit des Weizenmehles. I. Biochem. Z. **280**: 1.
1938 Entwicklung der Theorie der Bromatwirkung (1935–38). Mühlenlab. **8**: 201–208.
1938a Studies on the influence of chlorate, bromate, and iodate on the action of papain on casein. Compt. rend. Lab. Carlsberg, Sér. Chim. **22**: 246–254.
Kent-Jones, D. W.
1934 The practice and science of breadmaking. Northern Publishing Co., Liverpool.
1939 Modern cereal chemistry (3rd ed.). Northern Publishing Co., Liverpool.
Kohman, E. F., and Sanborn, N. H.
1937 Vegetable reduction of dehydroascorbic acid. Ind. Eng. Chem. **29**: 1195–1199.
Krebs, H. A.
1938 Intermediary hydrogen-transport in biological oxidation. In *Perspectives in Biochemistry*, Cambridge Univ. Press.
Lipmann, F.
1936 Hydrogenation of vitamin B_1. Nature **138**: 1097.
Maschmann, E., and Helmert, E.
1934 Über die Aktivierung des "Papains" durch Vitamin C—Eisen und seine Hemmung durch Vitamin C. Z. physiol. Chem. **223**: 127 + 135.
Melville, J., and Shattock, H. T.
1938 The action of ascorbic acid as a bread improver. Cereal Chem. **15**: 201–205.

Morgan, E. J., and Friedmann, E.
1938 Interaction of maleic acid with thiol compounds. Biochem. J. **32**: 733–742.
1938a Maleic acid as inhibitor of enzyme reactions induced by SH-compounds. Biochem. J. **32**: 862–870.
Nelson, E. K., and Hasselbring, H.
1931 Some organic acids of wheat plants. J. Amer. Chem. Soc. **53**: 1040–1043.
Neumann, M. P.
1929 Brotgetreide und Brot. Verlag Parey, Berlin.
Oppenheimer, C., and Stern, K. G.
1939 Biological oxidation. Nordemann Publishing Co., Inc., New York.
Pulkki, L. G., and Puutula, K.
1938 Über die Bildung und Verteilung der Askorbinsäure im Weizen während der Keimung. Z. ges. Getreidew. **25**: 149–153.
Purr, A.
1935 The reversible inactivation of papain and cathepsin. Biochem. J. **29**: 5–12.
Read, J. W., and Haas, L. W.
1938 Further studies concerning potassium bromate and enzyme activity. Cereal Chem. **15**: 59–68.
Rupp, E., and Bailey, C. H.
1937 The effects of protease increments on the plasticity of doughs. Cereal Chem. **14**: 496–510.
Sandberg, M., and Brand, E.
1925 Papain lipase. J. Biol. Chem. **64**: 59–70.
Schöberl, A., and Fischer, M.
1939 Über Schwefelgehalt und Aktivierbarkeit von Papain. Biochem. Z. **302**: 310–331.
Schultz, A., Atkin, L., and Frey, C. N.
1937 A fermentation test for Vitamin B_1. J. Amer. Chem. Soc. **59**: 948.
1939 Fermentation of maltose in the dough. Cereal Chem. **16**: 648–651.
1939a The vitamin B_1 content of wheat, flour and bread. Cereal Chem. **16**: 643–648.
Sherman, H. C.
1928 Chemistry of food and nutrition. Macmillan Co., New York.
Straub, F. B.
1937 Dehydrasekupplungen in der C_4- Dicarbonsäurekatalyse. Z. physiol. Chem. **249**: 189–199.
Sullivan, B., Near, C., and Foley, G. H.
1936 The harmful action of wheat germ on the baking quality of flour and the constituents responsible for this effect. Cereal Chem. **13**: 453–462.
Szent-Györgyi, A. v.
1937 Studies on biological oxidation and some of its catalysts. Eggenberger-sche Buchhandlung, Budapest.
1938 Oxidation and fermentation. In *Perspectives in Biochemistry*. Cambridge Univ. Press.
1938a L'oxydation biologique. Bull. soc. chim. biol. **20**: 846–860.
Triebold, H. O., Webb, R. E., and Rudy, W. J.
1933 A chemical study of rancidity. Cereal Chem. **10**: 263–276.
Weidenhagen, R.
1936 Beziehungen zwischen Vitamin C und enzymatischen Kohlenhydratspaltungen. Angew. Chem. **49**: 470.
Willard, J. T., and Swanson, C. O.
1913 The influence of certain substances upon the baking qualities of flour. Kansas Agr. Exp. Station Bul. 190.
Williams, R. R.
1939 Cereals as a source of vitamin B_1 in human diets. Cereal Chem. **16**: 301–309.
Wurmser, R.
1935. L'électroactivité dans la chimie des cellules. Actualités scientifiques et industrielles, No. 244. Paris.
Ziegler, E.
1940 Dough improvement studies. I. Cereal Chem. **17**: 460–468.
1940a Dough improvement studies. III. Cereal Chem. **17**: 556–564.

THE DETERMINATION OF TOTAL BETA-AMYLASE IN BARLEY AND BARLEY MALT[1]

S. R. SNIDER

U. S. Department of Agriculture,[2] Washington, D. C.

(Received for publication July 5, 1940)

Recent contemporary workers propose that the total saccharifying power in barley may be estimated by digesting the ground barley with papain. Myrbäck and Myrbäck (1933, 1936) attribute the increase in beta-amylase activity during germination to the proteolytic release of insoluble amylase bound to the protein molecule.

Chrzaszcz and Janiki (1933, 1936) hypothesize that certain "eleuto-substances" are formed during germination that nullify or counteract the inhibitory effect of a "sisto-substance" on beta-amylase activity.

Hills and Bailey (1938) concur with Myrbäck and Myrbäck and further conclude that the difference in the apparent increase in total beta-amylase during germination as measured by the rate of maltose production is in reality due to the increase in alpha-amylase activity.

Chrzaszcz and Swiatkowska (1937) in later investigations with hydrogen sulfide and papain conclude that hydrogen sulfide in conjunction with papain liberates a strongly bound component of beta-amylase that is not liberated by papain alone. Sallans and Anderson (1938) are not in complete agreement with this view, and present considerable data to support the opinion that Chrzaszcz and others used a grade of papain of inferior strength that was simply activated by hydrogen sulfide. This reasoning seems appropriate as it was stated that the papain used in their experiment was 1 : 100 in strength. In their recent study of the hydrogen sulfide-papain procedure, Myrbäck and Örtenblad (1939) conclude that the results of Chrzaszcz and others were distorted through amylase poisoning, probably by water containing copper salts.

It has not as yet been conclusively shown that the ultimate amount of total saccharifying activity has been estimated by any of the recent procedures employed. There is no evidence in the data of Hills and Bailey (1938) that steeping, germinating, and rootlet losses were considered when the total saccharifying power found in the barley was compared with the total saccharifying power found in the corresponding green malt. Chrzaszcz and Swiatkowska (1937) showed a maximum amount of saccharifying activity estimated under certain specific condi-

[1] Presented at the annual meeting of the American Society of Brewing Chemists, held in New York City, May 24, 1940.

[2] Joint study between Bureau of Plant Industry and Agricultural Marketing Service.

tions. They did not prove, however, that the entire amount of beta-amylase had been released and estimated.

Analyses for total saccharifying activity of barleys and green malts of the barleys, malted in the Grain Research Laboratory, Agricultural Marketing Service, Washington, D. C., in all cases indicated the presence of more beta-amylase in the green malt than in the barley, even when expressed in terms of the barley. These results may be interpreted as indicating one of two probabilities, that the analytical method employed did not estimate the entire amount of saccharifying activity in the barley, or that other enzymatic activity in the green malt such as alpha-amylase is erroneously estimated as beta-amylase additional to the total amount found in the barley.

In the earlier phases of this research, many barley and malt extracts were treated with papain and hydrogen sulfide at variable concentrations, times, temperatures, and hydrogen-ion concentrations, with little success. In every instance the result obtained by the use of papain alone was higher than those obtained by any papain-hydrogen sulfide combination.

The experiments discussed in this paper were performed for the purpose of developing a procedure providing optimum conditions of activation, hydrogen-ion concentration, time, and temperature for the extraction of the total beta-amylase in barley and malt, and indicating whether or not the last vestige can be released and estimated.

Barley Sample

A bulk sample of sound barley was finely ground and sieved to remove large particles and bran coat, in order to minimize variability within the sample. That portion passing through a No. 35 U. S. standard sieve was used in all subsequent experiments, and is designated as experimental sample No. 1. The papain was also sieved to remove lumps that might interfere with uniform mixing. A teaspoonful of 60-mesh alundum was added to all mixtures to prevent the papain from balling and to aid in obtaining a uniform paste from the mixture, before adding the entire amount of the extraction solution.

Papain

The selection of a satisfactory papain is most important, as commercial grades vary considerably in enzymatic strength. Three commercial samples, represented as 1:200 in strength, were selected and analyzed according to the method of Balls and Hoover (1937). One percent solutions of these samples, with and without cysteine, were used for the extraction of experimental barley sample No. 1. Diastatic power of the extracts was determined and the values are recorded in Table I.

Papain No. 1 and No. 2 are about standard strength for commercial grades. Papain No. 3 is unsatisfactory, as is evident from the results shown in the table. The use of cysteine in these experiments will be explained under "activation."

TABLE I

COMPARISON OF THE RELATIVE ACTIVITY OF THREE COMMERCIAL PAPAINS OF VEGETABLE ORIGIN

Experimental Sample No. 1

200 cc. of 5% extract for 20 hours at 20°C.

Papain				Cysteine HCl (neutralized)	Total saccharifying power	
No.	Identity	Amount	Activity units of Klim [1]		A.S.B.C. method	FeCy method
		g.		*g.*	°*L.*	°*L.*
1	Unknown	2	0.38	0	87.4	87.8
		2		1	82.8	85.4
		2		2	82.8	86.1
2	Ceylon	2	0.41	0	86.2	88.9
		2		1	84.6	88.3
		2		2	82.8	89.4
3	Mexican	2	0.06	0	66.1	71.5
		2		1	69.1	74.6
		2		2	72.1	76.5

[1] According to the method of Balls and Hoover (1937).

Time and Temperature for Extraction

Extractions were made at variable concentrations of barley and of papain, at variable temperatures and periods of time with and without toluene. The results of these experiments are shown in Table II.

An examination of the data in Table II shows: that 24 hours or thereabout is required to release the maximum amount of beta-amylase when papain alone is used; that no more beta-amylase can be obtained by prolonging the extraction time to 48 hours; and that a one percent solution of papain is sufficient for this purpose. It also appears that the addition of toluene to preserve the extract is not necessary if extractions are made at 20°C. Some extractions (not reported in Table II), made at room temperatures of 25°C. to 30°C. for 20 to 24 hours, showed slight evidence of fermentation when toluene was not used, and that the diastatic values were slightly lower than those of the preserved solutions. Briefly, it appears that the maximum yield can be obtained by extracting with one percent solutions of papain for 24 hours at 20°C. There is some evidence in the small amount of data shown that the same extraction could be accomplished in 3 hours at 40°C.

TABLE II

THE EFFECT OF VARIABLE CONCENTRATIONS OF BARLEY AND PAPAIN AT VARIABLE TIME AND TEMPERATURE

Barley Sample No. 1

Test No.	Barley	Papain	Water	Temperature	Time	Toluene	Diastatic power
	g.	*g.*	*cc.*	°*C.*	*hrs.*	*cc.*	°*L.*
1	25	3	150	20	24	4	89.2
2	25	4	200	20	24	4	88.9
3	10	2	200	20	24	4	90.5
4	10	2	200	20	24	–	89.5
5	10	2	200	20	48	–	88.2
6	10	2	200	20	48	4	87.6
7	10	4	200	20	24	–	88.0
8	10	3	200	20	24	–	88.6
9	10	2	200	20	24	–	89.5
10	10	1	200	20	24	–	87.5
11	10	0.5	200	20	24	–	85.1
12	10	0	200	20	24	–	25.6
13	10	4	200	20	3	–	85.3
14	10	3	200	20	3	–	83.8
15	10	2	200	20	3	–	82.9
16	10	1	200	20	3	–	78.3
17	10	0.5	200	20	3	–	72.9
18	10	0	200	20	3	–	24.0
19	10	4	200	40	3	–	86.5
20	10	2	200	40	3	–	89.4
21	10	1	200	40	3	–	80.8
22	10	0.5	200	40	3	–	77.9

Five percent extracts of barley and malt were made in all succeeding experiments, for convenience in a comparison of diastatic activity values by an accepted standard method.

Activation

It is generally known that the activity of papain can be accelerated by activation with hydrogen sulfide, hydrocyanic acid, cysteine, or glutathione. The initial trial experiments with hydrogen sulfide-papain combinations in this research confirmed the general opinion that it is an unsatisfactory agent for use when applied to the substrates of barley and malt. The hazard involved discourages the use of hydrocyanic acid for routine analytical purposes. Cysteine is an excellent activator of papain if complete oxidation of the sulfhydryl group is not effected during the course of activation. Glutathione does not possess any advantage over cysteine and it is too expensive for practical use; furthermore, cysteine is a constituent of the glutathione molecule. The use of cysteine as the activator facilitates the preparation of the extraction solution.

Very stable, although strongly acid, solutions of cysteine may be prepared with c. p. cysteine hydrochloride. When this solution is neutralized, as required, its stability gradually diminishes. Prepared stock solutions of cysteine hydrochloride deteriorated overnight, when neutralized, with the subsequent formation of cystine, which has no activating effect on papain. The stability of acid and neutral solutions of cysteine is shown in Table III.

TABLE III

STABILITY OF ONE PERCENT SOLUTIONS OF CYSTEINE-HCl AND CYSTEINE-HCl, NEUTRALIZED

Solution No.	Days standing before testing	Solution concentration in parts					
		1:100		1:1000		1:10000	
		Time [1]	Color [2]	Time [1]	Color [2]	Time [1]	Color [2]
		min.		*min.*		*min.*	
1	0	6	Deep purple	6	Pink	6	Yellow
		17	Green brown	17	Yellow	–	—
		24	Yellow	–	—	–	—
2	1	7	Deep purple	7	Pink	7	Yellow
		16	Green brown	16	Yellow	–	—
		25	Yellow	–	—	–	—
3	3	6	Deep purple	6	Pink	2	Yellow
		15	Light brown	15	Yellow	–	—
		22	Yellow	–	—	–	—
4	20	5	Deep purple	5	Pink	–	—
		15	Light brown	15	Yellow	–	—
		21	Yellow	–	—	–	—
1a [3]	1	12	Dark brown	12	Yellow	–	—
		20	Yellow	–	—	–	—
2a [3]	3	12	Light brown	2	Yellow	–	—
		17	Yellow	–	—	–	—

[1] Spot plate test, reading after adding nitroprusside reagent.
[2] Cysteine is completely oxidized to cystine when the characteristic yellow color of the mixed reagents is attained.
[3] Solutions 1 and 2, respectively, neutralized.

It is important to know the extent of cysteine oxidation during the extraction and filtration period, in order to ascertain the minimum amount of cysteine necessary to insure complete activation during the entire extraction period.

Cysteine may be detected in the presence of cystine by the nitroprusside reaction. A simple qualitative test to check the stability of the — SH group in extracts after the extract has been filtered and diluted, is to treat about 10 drops of the test solution on a spot plate with 5 to 10 drops of ammonium hydroxide (the solution must be alkaline) and 3 to 5 drops of sodium nitroprusside. Cysteine develops a purple color that varies in intensity with the concentration of cysteine present and that slowly fades as the cysteine is oxidized to cystine. One part of cysteine in 10,000 gives a distinct color reaction. It was observed that

diluted extracts 1:10, tested for cysteine stability, gave lower beta-amylase values when the characteristic color faded completely in less than 10 minutes.

In all experiments the cysteine hydrochloride was neutralized before using. Solutions 1a and 2a, Table III, are the neutralized solutions of 1 and of 2, respectively, that have been allowed to stand for 1 and 3 days respectively before testing by the nitroprusside method.

Extracts of experimental barley sample No. 1, with variable amounts of papain and cysteine, with and without citrate buffer, were tested for cysteine stability and diastatic power. The results are shown in Table IV. Test No. 6 gave a weak nitroprusside reaction and a correspondingly low diastatic power. The optimum ratios of papain and cysteine hydrochloride appear to be between 0.6 and 0.8 g. each for an extraction of 10 g. of barley.

TABLE IV

THE EFFECT OF ACTIVATION OF PAPAIN WITH CYSTEINE IN THE EXTRACTION SOLUTION, 3 HOURS AT 20°C.

Test No.	Papain	Cysteine HCl	Citrate buffer	pH of extract	Nitroprusside reaction	Diastatic power
	g.	*g.*				°*L.*
1	2.0	2.0	0	5.8	Deep purple	88.6
2	1.0	1.0	0	5.8	"	89.4
3	0.8	0.8	0	5.8	"	91.2
4	0.6	0.6	0	5.8	Purple	92.3
5	0.4	0.4	0	5.8	"	91.4
6	0.2	0.2	0	5.8	Faint purple	80.5
7	2.0	2.0	0.02*M*	5.0	Deep purple	82.5
8	1.0	1.0	0.02*M*	5.0	"	86.2
9	0.8	0.8	0.02*M*	5.0	"	87.1
10	0.6	0.6	0.02*M*	5.0	Purple	85.1
11	0.4	0.4	0.02*M*	5.0	"	86.0
12	0.2	0.2	0.02*M*	5.0	"	86.4
13	2.0	0.5	0	5.8	"	84.9
14	1.0	0.5	0	5.8	"	87.8
15	0.8	0.5	0	5.8	"	86.8
16	0.6	0.5	0	5.8	"	87.3
17	0.4	0.5	0	5.8	"	87.1
18	0.2	0.5	0	5.8	"	86.8

Effect of the Hydrogen-Ion Concentration

The effect of buffering the extraction solution for optimum activity of papain and cysteine was studied. Papain is most active in isoelectric solutions with optimum activity occurring at the isoelectric point of the protein substrate. Since there is no common isoelectric point for the proteins of barley, any arbitrarily chosen isoelectric point within the

isoelectric zone of the amylase-binding proteins should satisfy the conditions for optimum activity of papain.

Murray (1933) found that citrate buffers were superior to acetate and phthalate buffers for pH control of substrates subjected to the action of papain. Other conclusions were: that the citrate effect was a salt effect, extending the pH zone of activity, and that the activating effect of citrate was additive to that of other activators. It is known that citrate salts fix heavy metals. The presence of copper salts, for instance, in the steep waters would result in the inhibition of enzymatic activity. Weidenhagen (1936) found that vitamin C (ascorbic acid), even in minute quantities, reduced amylase activity, but that citric acid acted to a certain degree as an inhibitor to vitamin C.

In view of these useful properties of citrate salts for this type of enzymatic reaction, the importance of employing a citrate buffer, if for nothing more than as a precautionary measure, cannot be ignored. Certainly no ill effects attributable to the use of a citrate buffer could be observed in any of these experiments.

Combinations of papain-cysteine-treated extracts in the presence of citrate buffer at various pH and molarity were analyzed. Because of the difficulty of preparing citrate buffers at pH 7.0 phosphate buffer solutions were substituted for extractions at that hydrogen-ion concentration. The results are given in Table V, in which extracts without buffers are compared with the buffered extracts. It appears that opti-

TABLE V

EFFECT OF THE HYDROGEN-ION CONCENTRATION ON THE ESTIMATION OF TOTAL SACCHARIFYING POWER IN BARLEY AND MALT EXTRACTS

Experimental Sample No. 1

10 g. extracted with 200 cc. solution for 3 hours at 20°C. Extract Nos. 1, 2, and 3 contain 600 mg. of papain and 600 mg. of cysteine-HCl. Extract Nos. 4, 5, and 6 contain 800 mg. of papain and 800 mg. of cysteine-HCl.

Extract No.	Molarity	Buffer solutions (extract)									
		Citrate pH 5.0		Citrate pH 6.0		Citrate pH 6.5		Phosphate pH 7.0		None	
		D.P.[1]	pH	D.P.[1]	pH	D.P.[1]	pH	D.P.[1]	pH	D.P.[1]	pH
		°L.		°L.		°L.		°L.		°L.	
1	0.02	85.1	5.0	86.4	5.6	89.7	6.2	84.7	6.7	89.5	5.8
2	0.05	87.4	5.0	89.2	5.8	90.5	6.3	88.4	6.8	—	—
3	0.10	—	—	89.2	5.9	90.1	6.4	89.4	6.9	—	—
4	0.02	87.1	5.0	86.8	5.6	90.1	6.2	85.5	6.7	91.2	5.8
5	0.05	—	—	87.1	5.8	90.8	6.3	88.5	6.8	—	—
6	0.10	—	—	86.4	5.9	91.9	6.4	90.5	6.9	—	—

[1] Diastatic power, degrees Lintner.

mum results are obtained with 0.05*M* citrate buffer concentration at pH 6.5, although differences of less than 3° Lintner are really not significant. The use of a citrate buffer is precautionary, as has been explained.

Oxidation and Reduction Studies with Phenylhydrazine

Phenylhydrazine is regarded by Bergmann (1936) and Bergmann and Ross (1935) as an activator of natural papain. It is presumed by Balls and Hoover (1937) that this activation is due to the reduction of the natural activator, analogous to its reduction of methylene blue. A few miscellaneous trial experiments were performed to observe the influence of phenylhydrazine in the presence of the various reagents. These results are shown in Table VI. It is apparent that phenylhydrazine does not materially assist cysteine in the reduction of papain. The activating effect of citrate buffer is considerably impaired when phenylhydrazine is present. There appeared to be an actual inhibition of diastatic activity when phenylhydrazine was used in the absence of cysteine and buffer solution. (The free diastatic power of the barley sample was 25.6° Lintner.) It is assumed from the information accumulated from these experiments that sufficient cysteine was present to reduce completely the papain and carry the reaction to completion.

TABLE VI

THE INFLUENCE OF PHENYLHYDRAZINE ON THE REAGENTS USED IN THE EXTRACT FOR DETERMINING TOTAL SACCHARIFYING POWER

Experimental Sample No. 1

200 cc. of 5% extract for 20 hours at 20°C.

Extract No.	Papain [1]	Cysteine HCl [2]	Solution concentration			Color reactions		D.P.[6]
			Citrate buffer [3]	Solution pH	Phenylhydrazine [4]	Methylene blue [5]	Nitro prusside	
	mg.	*mg.*						
1	600	600	0	—	0	Faint greenish blue	Purple	86.6
2	600	600	.05*M*	6.3	0	"	"	87.4
3	600	600	0	—	.05*M*	Light blue restored	"	86.2
4	600	600	.05*M*	6.3	.05*M*	"	"	88.5
5	600	0	0	—	0	Blue	—	86.3
6	600	0	.05*M*	6.3	0	Blue	—	89.2
7	600	0	0	—	.05*M*	Colorless	—	5.2
8	600	0	.05*M*	6.3	.05*M*	Colorless	—	35.7

[1] Dry powder added to ground barley.
[2] Neutralized with *n*-NaOH to pH 6.5±.
[3] Final molarity of buffer in the extract.
[4] Final molarity of phenylhydrazine in the extract.
[5] 0.25 cc. of saturated solution of methylene-blue added. Color noted at the beginning and end of the extraction period.
[6] Diastatic power, degrees Lintner.

Development of a Procedure

The data accumulated in all of the preceding experiments seem to justify the assumption that (1) as much, if not more, saccharifying activity can be obtained from ungerminated barley by the use of 0.6 g. to 0.8 g. of cysteine and equal amounts of papain in three hours' extraction as can be obtained with 2 g. of papain alone in 24 hours' extraction, (2) that citrate buffer, 0.05 molar concentration, pH 6.4 to pH 6.7, may be used as a precautionary measure to prevent any possible inhibition due to heavy metals or vitamin C, without affecting the results, (3) that temperatures higher than 20°C. do not give a higher yield, and (4) that the addition of toluene as a preservative is not necessary for the short-time extraction. These indications would suggest the following short method for the determination of total diastatic power in ungerminated barley.

Reagents

Citrate buffer solution: 0.2*M*, pH 6.4–6.6.

Solution a: Dissolve 82.016 g. of c.p. citric acid ($C_6H_8O_7.H_2O$) in 400 cc. of carbonate-free 1.0*N* NaOH, add 1 cc. of toluene for preserving, and dilute to 1 liter. The pH of this solution should be 4.96.

Solution b: Mix 530 cc. of solution *a* with 470 cc. of 0.2*N* NaOH. The pH of this solution should be about 6.6.

Phosphate buffer solution: 0.1*M*, pH 8.3. Dissolve 14.2 g. of c.p. anhydrous Na_2HPO_4 in CO_2-free distilled H_2O, add 1 cc. of toluene, and make up to 1 liter.

Cysteine-HCl solution: Dissolve 18.75 g. of c.p. cysteine-HCl in distilled H_2O and make up to 500 cc. Twenty cubic centimeters of this solution should require 4.5 cc. of 1.0*N* NaOH for neutralization.

Procedure

For barley: Pipette 20 cc. of cysteine-HCl solution into a 200-cc. volumetric flask and neutralize with 4.5 cc. of 1.0*N* NaOH, add 50 cc. of citrate buffer solution *b* and make up to 200 cc. with distilled H_2O. Test this solution with brom-thymol blue on a spot plate. The pH should fall within the range of 6.4 to 6.7.

Weigh out 10 g. of finely ground barley into a 250-cc. Erlenmeyer flask. To this, add 750 mg. of standard-strength papain and a teaspoonful (approximately 15 g.) of 60-mesh alundum. To this mixture add a small portion of the buffered cysteine solution to obtain a pasty consistency. Swirl the flask until all lumps are broken up. Add the remainder of the extraction solution, stopper the flask, and immerse in a water bath at 20°C. This extract should be agitated at intervals of 15 to 20 minutes. After 2½ hours, filter the extract through 18 ½ cm. S and S fluted filter paper. Cover the funnel with a watch glass to arrest atmospheric oxidation and evaporation. Reject the first 50 cc. of the

filtrate. If it is preferred to extract 25 g. of barley, the same ratio of reagents applies. Pipette 20 cc. of the extract (or 10 cc. if the diastatic power exceeds 135° Lintner) into a 100-cc. volumetric flask and dilute to 100 cc. At this stage proceed according to the method of Anderson and Sallans (1937) as outlined by Laufer, Schwarz, and Laufer (1938) for the determination of diastatic power by the ferricyanide method.

The procedure for green malts or kilned malts is the same unless it is desired to estimate the saccharifying activity of beta-amylase only. In this case the Ohlsson (1926, 1930) acidification technique for differential inactivation of alpha-amylase is employed. Ohlsson's method is not quantitative but may be used in studies pertaining to experimental malting. Otherwise the diastatic-power values of green malt represent the combined activities of alpha-amylase and beta-amylase. If a differential estimation is desired proceed in the same manner as in the analysis of barley up to the filtration of the extract. Pipette three aliquots of 20 cc. or 10 cc. (as the case may require) into a 100-cc. volumetric flask and into two 100-cc. Erlenmeyer flasks. Immerse the volumetric flask in an ice-water bath, using lead rings so that the flask may be almost entirely immersed. If feasible place the ice-water bath within the cooling compartment of a refrigerator; otherwise the bath must be placed in the coldest available spot within the refrigerator.

Predetermine electrometrically, on one of the aliquots, the amount of 0.1*N* HCl necessary to bring the extract to pH 3.3. The progress of this titration may be checked by titrating incrementally and testing the solution on a spot plate with brom-phenol blue as pH 3.3 is approached, to avoid the necessity of several electrometric determinations. When the temperature of the extract in the ice-water bath is reduced to 0°–4°C., add the predetermined amount of 0.1*N* HCl to the test solution and return the bath with the flask to the refrigeration compartment for 15 minutes.

To the third aliquot add the same amount of HCl as used in the other aliquots and determine electrometrically the amount of phosphate buffer solution necessary to restore the solution to pH 6.7. Remove the test solution from the refrigerator after 15 minutes of the acid treatment and add the predetermined amount of phosphate buffer solution required for neutralization. Dilute the solution to 100 cc. and proceed with the determination for diastatic power.

If desired, this diluted test solution may be tested on a spot plate for cysteine stability by treating 10 drops with 5 drops of 5% solution of sodium nitroprusside and 5 drops of 10% ammonium hydroxide. If the purple color of the mixture does not fade out completely in less

than 10 minutes, optimum activation of papain by the sulfhydryl group of cysteine has been maintained during the entire extraction period.

Application of the Method

The experiments thus far reported were made with one sample of barley of low diastatic power. Six varieties of barley ranging from low to high diastatic power were then selected and analyzed for total diastatic power by the old method (that is, digestion with a one percent solution of papain for 20 to 24 hours) and by the short procedure. The results are shown in Table VII. The Lintner values obtained by the modified

TABLE VII

COMPARISON OF TOTAL DIASTATIC POWER IN BARLEYS, DETERMINED BY THE MODIFIED METHOD AND BY THE OLD METHOD

Sample No.	Variety	Old method		Modified method	
		D.P.[1]	pH of extract	D.P.[1]	pH of extract [2]
2	Atlas	79.8	5.5	84.0	6.5
3	Spartan	74.8	5.5	73.8	6.4
4	Velvet	109.6	5.6	110.0	6.5
5	Trebi	110.8	5.6	111.6	6.5
6	Oderbruker	157.6	5.5	164.0	6.4
7	Peatland	184.0	5.5	189.8	6.4

[1] Diastatic power in degrees Lintner.
[2] Extract buffered with citrate buffer, pH 6.7.

method are slightly higher than those of the old method. This is not definitely significant, as comparison is made with only a small number of samples. The results on green malts, however, are quite significant.

Nine samples of barley were malted until germination was completed according to recognized standard malting procedure. The green malt was removed from the germination cabinet and rapidly air-dried in a drying cabinet at 93°F. These samples of malt were analyzed for total diastatic power by the old method and by the short method. The green malts were analyzed for alpha-amylase activity by the method of Ehrnst, Yakish, and Olson (1939). Extracts for total beta-amylase of the green malt by the short method were treated for alpha-amylase destruction, with 0.1*N* HCl at 0°C. for 15 minutes and analyzed for diastatic power. The malting losses were determined during the course of malting. The original barley samples were analyzed for total saccharifying activity by the short method. The Atlas barleys, samples No. 11 and No. 12, were grown under unusual soil and climatic conditions, which may account for the exceptionally high diastatic values for this variety. The results are recorded in Table VIII.

TABLE VIII

COMPARISON OF THE METHODS AND APPLICATION OF THE MODIFIED METHOD IN THE ANALYSIS OF GREEN MALTS

Sample No.	Barley variety	Total saccharifying power, green malt, dry basis			Malting loss—steeping, germinating, and rootlet	Total saccharifying power, green malt corrected for malting losses to basis of barley			Total saccharifying power, barley	Variable [2]		Dextrinization values—modified Wohlgemuth method [2]—green malt, dry basis
		Papain method [1]	Papain-cysteine method [3]	Papain-cysteine method [2] less alpha-amylase		Papain method [1]	Papain-cysteine method [3]	Papain-cysteine method [2] less alpha-amylase	Papain-cysteine method [3]	Green malt vs. barley, total saccharifying power	Green malt, total beta-amylase less alpha-amylase vs. barley	
		°L.	°L.	°L.	%	°L.	°L.	°L.	°L.	°L.	°L.	
8	Atlas	76.9	79.8	71.2	11.0	68.5	71.0	63.4	58.5	21.3	4.9	45.5
9	"	89.5	93.2	86.5	11.0	79.7	82.9	76.2	79.4	13.8	−3.2	48.8
10	"	75.5	81.7	76.5	11.3	67.0	72.5	66.9	63.9	17.8	3.0	49.8
11	"	168.6	180.6	130.0	10.7	151.2	161.3	116.3	122.5	58.1	−6.2	77.8
12	"	166.0	180.0	146.5	10.2	149.1	161.6	131.6	124.0	56.0	7.6	82.1
13	Velvet	259.6	272.0	201.1	11.0	222.1	244.0	179.0	180.7	91.3	−1.7	114.7
14	Wisconsin 38	277.8	288.4	220.0	10.0	250.0	259.6	198.0	203.1	85.3	−5.1	132.3
15	Manchurian	251.3	260.0	184.4	11.5	222.4	230.1	163.2	172.8	87.2	−9.6	119.5
16	Odessa	224.0	228.7	183.2	9.3	203.2	207.4	166.2	151.1	77.6	15.1	105.4

[1] Five % extracts with 1% solution of papain for 24 hours at 20°C.
[2] Modified method.
[3] Method of Ehrnst, Yakish, and Olson (1939).

There is a significantly higher saccharifying power by the short method than by the old method, determined in terms of degrees Lintner. The same extracts of green malt show considerably more diastatic power than the corresponding barleys after correction for malting losses. The extracts of green malt in which the alpha-amylase was destroyed, however, show nearly the same values, after correction for malting losses, as the barleys.

The difference in total diastatic power between barley and green malt, in which the alpha-amylase has not been destroyed, does not quantitatively correlate with the dextrinization values.

There is a relation, however, between the total and apparent increase in degrees Lintner, presumably produced by beta-amylase when the alpha-amylase has not been destroyed, and the dextrinizing power determined directly by the method of Ehrnst, Yakish and Olson (1939).

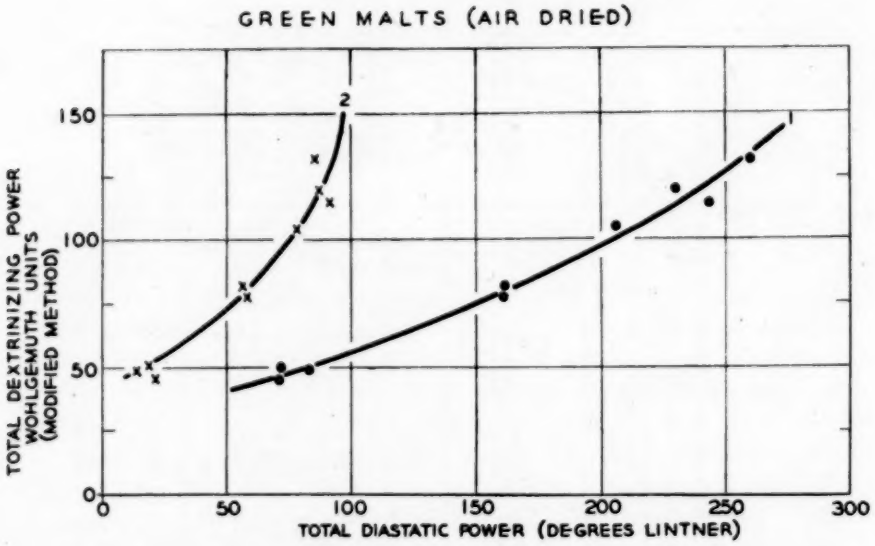

Fig. 1. Curve 1: relation of total diastatic power to dextrinizing power. Curve 2: apparent increase in saccharifying power of green malt over that of the corresponding barley due to the presence of alpha-amylase, compared with dextrinization values.

This is shown in Figure 1. These results indicate that the increase in maltose production in a starch substrate treated with malt extract over that treated with the corresponding barley extract is not entirely due to the additive alpha-amylase activity, and that the combined saccharifying power of alpha-amylase and beta-amylase exceeds the sum of their separate activities.

Freeman and Hopkins (1936) observed that the limit of hydrolysis with mixed amylases is only 10% to 12% greater than with single amylases of similar total activity, which suggested that at least half of the starch constitutes a substrate common to both enzymes.

It is demonstrated in Figure 1 that the effect of alpha-amylase on saccharifying activity becomes less apparent as the concentration of the amylases is increased. Similarly, Sandstedt, Kneen, and Blish (1939) found that the rate of dextrinization increased up to a certain point as the ratio of beta-amylase to alpha-amylase in the starch enzyme mixture increased.

Summary

The total beta-amylase of barley, green malt, and malt can be extracted in 3 hours with a papain-cysteine combination in the presence of a citrate buffer.

The saccharifying activity in barley and malt is more conveniently estimated by the proposed short-time extraction method than by extraction for 20 hours with papain alone.

The apparent increase in total beta-amylase in green malt over that of the corresponding barley appears to be due chiefly to the combined effects of alpha- and beta-amylase.

Papain used in the extraction mixture should be not less than 1:200 in strength.

The proposed method is useful for accurately predicting the total available beta-amylase in barley, and/or for gauging the efficiency of a malting procedure with respect to the production of beta-amylase from that barley.

Acknowledgments

The green malts used in these experiments were prepared by H. B. Dixon in the Grain Research Laboratory, Washington, D. C., in the course of investigational studies on the malting quality of barleys.

Literature Cited

Anderson, J. A., and Sallans, H. R.
1937 Determination of the diastatic power of malt in degrees Lintner by means of a ferricyanide reagent. Can. J. Research **C15**: 70–77.

Balls, A. K., and Hoover, S. R.
1937 The milk-clotting action of papain. J. Biol. Chem. **121**: 737–745.

Bergmann, M.
1936 The enzymes of papain and their activation. J. Biol. Chem. **114**: 717–726.

—— and Ross, W. F.
1935 The proteolytic systems of papain. J. Biol. Chem. **111**: 659–666.

Chrzaszcz, T., and Janiki, J.
1933 Eleutoamylase, eine Antisistoamylasesubstanz. Biochem. Z. **263**: 250–261.

1936 Amylase during the growth and ripening of grains. Biochem. J. **30**: 1298–1302.

Chrzaszcz, T., and Swiatkowska, W.
1937 Zur Festellung der wirklichen Amylasemenge in Gerstenmalz durch Pepton, Proteinasen und Schwefelwasserstoff. Biochem. Z. **290**: 225–234.

Ehrnst, L. E., Yakish, G. J., and Olson, W.
1939 A modification of the Wohlgemuth method for the determination of alpha-amylase and a comparison of this method with a viscosity method. Cereal Chem. **16**: 724–729.

Freeman, G. G., and Hopkins, R. H.
1936 The mechanism of degradation of starch by amylases. Biochem. J. **30**: 442–456.

Hills, C. H., and Bailey, C. H.
1938 The amylases of different barley varieties. Cereal Chem. **15**: 351–362.

Laufer, S., Schwarz, E., and Laufer, L.
1938 Determination of diastatic power of malt. American Brewer **71**: 25–27.

Murray, D. R. P.
1933 The activation of papain by cyanide and other substances. I. Biochem. J. **27**: 543–555.

Myrbäck, K., and Myrbäck, S.
1933 Über Gersten und Malzamylase. Biochem. Z. **258**: 158–171.
1936 Über freie und gebunden Amylase in der Gerste. Biochem. Z. **285**: 282–289.

Myrbäck, K., and Örtenblad, B.
1939 Amylasebestimmungen in Gerste. Enzymologia **7**: 176–181.

Ohlsson, E.
1926 On the two components of malt diastase. Compt. rend. trav. lab. Carlsberg **16**: 1–68.
1930 Über die beiden Komponenten der Malzdiastase, besonders mit Rucksicht auf die Mutarotation der bei der Hydrolyse der Stärke gebildeten Produkte. Z. für Physiol. Chem. **189**: 17–63.

Sallans, H. R., and Anderson, J. A.
1938 II. Saccharifying activities of barleys and malts and the correlations between them. Can. J. Research **C16**: 405–416.

Sandstedt, R. M., Kneen, E., and Blish, M. J.
1939 A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16**: 712–723.

Weidenhagen, R.
1936 Beziehungen zwischen Vitamin C und enzymatischen Kohlenhydratspaltungen. Z. Wirtschaftsgruppe Zukerindustrie. Tech. T. **86**: 482–494.

VALUE OF ASCORBIC ACID FROM VARIOUS SOURCES IN BAKING

J. F. FEASTER [1] and W. H. CATHCART [2]

(Read at the Annual Meeting, May 1940)

Breads including grapefruit juice in the formula are receiving consideration; thus the need for information regarding the value of added grapefruit juice from the viewpoints of nutritive values and baking properties becomes apparent. The vitamin C in grapefruit juice might enhance the nutritive value of bread. From available information (Cathcart, 1938) it also appears that grapefruit juice might be a valuable ingredient in bread from the standpoint of baking, possibly by acting as a valuable "dough conditioner." In order to obtain information on these two points, a preliminary investigation of grapefruit-juice bread was conducted.

General Discussion of the Problem

l-Ascorbic acid is a relatively labile compound under conditions which permit exposure to oxygen at elevated temperatures. Procedures employed in making bread permit considerable exposure to oxygen and require the use of elevated temperatures. In addition, flour contains an enzyme system which catalyzes the oxidation of *l*-ascorbic acid (Melville and Shattock, 1938). It follows that the *l*-ascorbic acid content of grapefruit-juice bread can not be calculated from the *l*-ascorbic acid content of the grapefruit juice added.

Jørgensen (1935) announced the discovery that *l*-ascorbic acid could act as a dough conditioner and that it has an effect similar to that of potassium bromate, an oxidizing agent. The action of *l*-ascorbic acid, like bromate, is said to involve an inhibition of the flour proteinases (Jørgensen, 1939). Melville and Shattock (1938) have shown that the enzyme ascorbic acid oxidase of flour catalyzes the oxidation of *l*-ascorbic acid to *l*-dehydroascorbic acid. The *l*-dehydroascorbic acid is a dough conditioner and, in general, is said to be equivalent to bromate on a weight-for-weight basis. Burian (1939) has found that *l*-ascorbic acid retards the action of proteolytic enzymes, but unlike potassium bromate has no effect on the viscosity of the proteins and their degradation products, and thus does not influence the physical properties of the dough. Because of this latter fact, Burian states that the chemical treatment of flours with *l*-ascorbic acid should not be overrated.

[1] American Can Company, Maywood, Illinois.
[2] American Institute of Baking, Chicago, Illinois.

Although it appeared that *l*-ascorbic acid in grapefruit-juice bread would be poorly retained, experiments were performed investigating the fate of this nutrient and the effect of ascorbic acid from various sources upon the baking qualities of bread.

It has been reported that grapefruit juice is a valuable ingredient for retarding mold growth; however, this phase of the problem was not investigated.

Investigation of *l*-Ascorbic Acid Stability

The *l*-ascorbic acid was determined by the 2, 6-dichlorophenolindophenol titration method modified to use a solution containing 2% metaphosphoric acid and 6% acetic acid (Bessey, 1938). The dye solution was standardized against an *l*-ascorbic acid solution which in turn was standardized against a standard iodine solution.

Since there is some doubt as to whether the increase in *l*-ascorbic acid which may be produced by preliminary treatment with hydrogen sulfide is due to reduction of *l*-dehydroascorbic acid or the formation of other reducing materials, this procedure was omitted. The *l*-ascorbic acid values reported do not include any *l*-dehydroascorbic acid which may have been present. *l*-Dehydroascorbic acid has antiscorbutic properties similar to *l*-ascorbic acid (Borsook and co-workers, 1937). However, in slightly acid or neutral solutions, *l*-dehydroascorbic acid is readily converted to diketogluconic acid which is devoid of antiscorbutic properties. Any antiscorbutic value due to *l*-dehydroascorbic acid which might be contained in the baked products at the time consumed can only be definitely established by bioassay methods.

The *l*-ascorbic acid content of the grapefruit juice was determined by diluting 5.0 ml. to 50.0 ml. with a solution of the extracting acid and titrating without further treatment. Since the *l*-ascorbic acid value of the canned grapefruit juice determined by titrating in a mixture of 2% metaphosphoric acid and 6% acetic acid was within 3% of the *l*-ascorbic acid value determined by titrating in 8% acetic acid, it appears that the iron content of this canned grapefruit juice did not materially affect the *l*-ascorbic acid value determined by titrating in the presence of metaphosphoric acid.

The bakery products were prepared for titration in the following manner: A 5.0-g. sample was ground to a smooth paste with sea sand and 5.0 ml. of the solution of metaphosphoric acid and acetic acid. This mixture was made up to 30 ml. with the extracting acid in a graduated centrifuge tube, thoroughly mixed, then centrifuged. A suitable aliquot (usually 15 ml.) of the supernatant liquor was diluted to 25 ml. and titrated with the standard dye solution.

The following ingredients were used for both the straight and sponge dough processes:

	Percent on basis of total flour 100%
Flour	100
Water (approx.)	62
Salt	2
Yeast	2
Milk solids	4
Corn sugar	5
Shortening	4
Malt extract	1

Grapefruit juice in the quantities desired was used to replace the water in the formula. The amount of sugar in the formula was adjusted according to the amount of grapefruit juice used and the amount of sugar in the juice. In the sponge and dough process the grapefruit juice was added at the dough stage. When *l*-ascorbic acid was used, it was added to the formula given, dissolved in part of the water. In the sponge and dough method it was added at the dough stage. Semicommercial breadmaking technique was used throughout the experiments.

The formula for the cake used in the experiments is as follows:

	Percent on basis of flour 100%
Flour	100
Sugar	120
Shortening	30
Whole eggs	50
Grapefruit juice	95
Lemon flavor	9.75
Sodium bicarbonate	0.62
Salt	3
Baking powder	3.75

Discussion of Results

From the data in Table I it appears that conventional-type white bread (mix No. 1, control) contains approximately 0.003 mg. of *l*-ascorbic acid per gram. However, it is very doubtful if this should be considered as a true *l*-ascorbic acid value. It probably represents the difference between a blank for the 2, 6-dichlorophenolindolphenol titration in a solution of the extracting acid and the blank for the titration in the extracting acid plus constituents, extracted from bread, other than *l*-ascorbic acid.

The *l*-ascorbic acid contents of doughs containing added sources of *l*-ascorbic acid (mixes 2, 3, 4, and 5) from the mixer or bench were considerably less than the corresponding *l*-ascorbic acid values for the mix calculated from the weight of the mix and *l*-ascorbic acid added as grape-

fruit juice or crystalline *l*-ascorbic acid. The mixing of bread beats considerable quantities of air into the dough. In addition, the report of Melville and Shattock (1938) demonstrates an enzyme system in flour which is capable of promoting the oxidation of l-ascorbic acid to *l*-dehydroascorbic acid, which acts in a manner similar to bromates. Hence the lower *l*-ascorbic acid contents of the doughs which were found as compared with the *l*-ascorbic acid contents calculated from the formulas of the mixes indicate partial oxidation of *l*-ascorbic acid during mixing.

TABLE I

l-Ascorbic Acid Content of Breads

Mix No.	Type of bread	Weight of mix	Source of *l*-ascorbic acid	*l*-Ascorbic acid [1]	Sample from	*l*-Ascorbic acid found [2]
		lbs.		*mg./gm.*		*mg./gm.*
1	Straight dough	28.9	Control (none)	—	Mixer	0.003
1	Straight dough	28.9	Control (none)	—	Bench	0.004
1	Straight dough	28.9	Control (none)	—	Baked	0.003
2	Straight dough	27.3	2.25 lbs. grapefruit juice	0.030	Mixer	0.010
2	Straight dough	27.3	2.25 lbs. grapefruit juice	0.030	Bench	0.010
2	Straight dough	27.3	2.25 lbs. grapefruit juice	0.030	Baked	0.004
3	Straight dough	26.3	4.50 lbs. grapefruit juice	0.063	Mixer	0.033
3	Straight dough	26.3	4.50 lbs. grapefruit juice	0.063	Bench	0.032
3	Straight dough	26.3	4.50 lbs. grapefruit juice	0.063	Baked	0.006
4	Sponge dough	27.0	3.72 lbs. grapefruit juice	0.046	Second mix	0.034
4	Sponge dough	27.0	3.72 lbs. grapefruit juice	0.046	Baked	0.007
4	Sponge dough	27.0	3.72 lbs. grapefruit juice	0.046	24-hour bread	0.003
5	Sponge dough	27.0	600 mg. *l*-ascorbic acid	0.049	Second mix	0.030
5	Sponge dough	27.0	600 mg. *l*-ascorbic acid	0.049	Baked	0.003
5	Sponge dough	27.0	600 mg. *l*-ascorbic acid	0.049	24-hour bread	0.003

[1] Calculated from *l*-ascorbic acid content of grapefruit juice, or from weight of *l*-ascorbic acid added.
[2] Found by titration.

The *l*-ascorbic acid values of the order of 0.003 to 0.007 mg. per gram (ascorbic acid values in the blanks were almost equally as high), or 0.085 to 0.198 mg. per ounce, found for the baked breads with added grapefruit juice or *l*-ascorbic acid, compared with *l*-ascorbic acid values of the order of 0.030 to 0.063 mg. per gram, or 0.85 to 1.78 mg. per ounce calculated from the added grapefruit juice or *l*-ascorbic acid, indicate that the major portions of the added *l*-ascorbic acid were oxidized by the time the loaves were baked and cooled. The addition of grapefruit juice to bread does not appear to be practical as a means of increasing the *l*-ascorbic acid content of bread to a point where bread may be relied upon as a source of *l*-ascorbic acid of appreciable value in supplying the human requirement for vitamin C, which is of the order of 30 milligrams of *l*-ascorbic acid per day. The results are not in agreement with the finding of Zavyalov and Zavyalov (1938) that *l*-ascorbic acid in concentrated extracts of briar roses or of coniferous

needles added to bread is retained in the extent of 60% to 70% of the added *l*-ascorbic acid.

Cake batter and baked cake were more easily prepared for *l*-ascorbic acid titration than were bread dough and baked bread.

Data presented in Table II indicate that batter of commercial-type layer cake contains no appreciable amounts of *l*-ascorbic acid. The *l*-ascorbic acid content of batter with added grapefruit juice was found to be 0.077 mg. per gram as compared with 0.078 mg. per gram calculated from the weight of the batter and the *l*-ascorbic acid content of the added grapefruit juice. The baked cake was found to contain 0.039 mg. per gram. The *l*-ascorbic acid content of the cake decreased during storage, until 24 and 48 hours after baking the *l*-ascorbic acid contents of 0.023 and 0.021 mg. per gram, respectively, were found.

TABLE II

l-Ascorbic Acid Content of Cake

Mix No.	Type of cake	Total wt. of mix	Source of *l*-ascorbic acid	*l*-Ascorbic acid [1]	Sample	*l*-Ascorbic acid found [2]
		lbs.		*mg./g.*		*mg./g.*
1	Layer	—	None	0.000	Batter	0.000
2	Layer	5.0	1.19 lbs. grapefruit juice	0.078	Batter	0.077
2	Layer	5.0	1.19 lbs. grapefruit juice	0.078	Baked	0.039
2	Layer	5.0	1.19 lbs. grapefruit juice	0.078	24-baked	0.023
2	Layer	5.0	1.19 lbs. grapefruit juice	0.078	48-baked	0.021

[1] Calculated from *l*-ascorbic acid content of grapefruit juice.
[2] Found by titration.

The better retention of *l*-ascorbic acid in cake, as compared with bread, may be associated with less intensive mixing, the use of relatively smaller quantities of flour (hence smaller quantities of ascorbic acid oxidase), and a somewhat shorter baking time with cake as compared to bread.

The cake made from the formula containing grapefruit juice was of high quality and pleasing flavor.

The Dough-Conditioning Value of *l*-Ascorbic Acid, *d*-Ascorbic Acid, and Grapefruit Juice

The above work shows that the *l*-ascorbic acid content of baked bread prepared from formulas containing synthetic *l*-ascorbic acid or grapefruit juice was negligible. This indicates that the inclusion of *l*-ascorbic acid in bread formulas does not increase materially the antiscorbutic properties of the baked product. Therefore it was decided

to include *d*-ascorbic acid, which has no antiscorbutic value, as well as its optical isomer, *l*-ascorbic acid (vitamin C), in the baking quality tests.

In the experimental procedure both laboratory and commercial baking tests were performed. The laboratory test was designed to determine the true baking quality of the flour (including the effect of other ingredients) without the aid of enrichening ingredients. The commercial test was used to show how the flour, etc., would behave in a commercial bakery. Enrichening ingredients such as sugar, milk, and shortening were used in making the commercial loaves. Many tests were made; however, only typical examples are given in the results herewith presented.

Results and Discussion

Results are not given on grapefruit juice, for in quantities furnishing approximately 5 mg. of *l*-ascorbic acid per 100 g. of flour [3] (approximately 15% grapefruit juice on the basis of flour), the dough-conditioning value of *l*-ascorbic acid was apparently overshadowed by the effect of the citric acid content. This probably is due to a decrease in the gas-retaining properties of the gluten. Smaller quantities might have been of value in conditioning the dough.

Recommendations for making grapefruit-juice bread are to use approximately 15% on the basis of flour as 100%. However, if grapefruit juice is to be used to increase the nutritive properties of the bread, the largest quantities possible should be used. Thus quantities varying from 7.5% to 30% were tried, and in all these concentrations it decreased volume and gave a more dense texture in proportion to the quantity used. With the straight-dough method it was almost impossible to produce a loaf with properties similar to the control loaf, even with the lowest percentage. The sponge and dough method (grapefruit juice put in at dough stage) yielded much better results; however, it was necessary to give the dough a minimum amount of fermentation time. In view of these results grapefruit juice would probably not be desirable as a regular ingredient in white bread, but can be used for producing a special product.

Table III shows typical results of the effect of potassium bromate, *l*-ascorbic acid, and *d*-ascorbic acid on bread. All loaves were scored relatively for external and internal characteristics (100 points being best score for each) and volumes measured.

The flour used had a definite bromate response as is shown by the results for potassium bromate in Table III. The *l*-ascorbic acid was just as effective as bromate in improving the bread, while *d*-ascorbic had

[3] Melville and Shattock (1938) showed that this quantity of *l*-ascorbic acid had a definite improving action on bread.

little effect. The *d*-ascorbic acid seemed to be detrimental except in quantities of 1.5 mg. per 100 g. flour. The same trend in results is shown by both the experimental and commercial loaves. The slight difference between the experimental and commercial loaves is, undoubtedly, due to a difference in formula—sugar, shortening, and milk being used in the commercial samples. Other experiments confirmed the results of Table III.

TABLE III

EFFECT OF BROMATE, *l*-ASCORBIC ACID, AND *d*-ASCORBIC ACID ON BREAD QUALITY
Quantities given in mg. per 100 g. flour. High grade unbleached and unmatured flour used

	Standard	$KBrO_3$				*l*-Ascorbic				*d*-Ascorbic			
	—	0.5	1.5	3.0	6.0	0.5	1.5	3.0	6.0	0.5	1.5	3.0	6.0
Experimental loaves:													
Volume in cubic inches	91.0	88.0	100.0	91.5	99.0	98.5	100.0	98.5	100.5	88.0	93.5	83.5	86.5
External score	94	98	99	93	97	100	100	100	99	91	96	91	89
Internal score	88	96	99	94	95	93	97	98	100	89	91	84	84
Commercial loaves:													
Volume in cubic inches	178.0	181.0	184.0	194.5	184.0	181.3	188.0	188.5	188.0	176.0	179.0	171.5	169.5
External score	92	94	100	98	98	93	96	97	97	85	90	88	87
Internal score	89	94	96	98	100	88	99	96	97	83	92	86	81

Experiments using a flour that was properly bleached and matured at the mill gave indications that potassium bromate was slightly different in its action from *l*-ascorbic acid. It was possible to add more *l*-ascorbic acid than potassium bromate without showing detrimental results; that is, the flours seemed to have greater tolerance to *l*-ascorbic acid than to potassium bromate. This might prove to be a slight advantage for *l*-ascorbic acid.

These results confirm the work of Jørgensen, Melville, and Shattock, and Burian. However, there was no indication that with the flours used (various grades) *l*-ascorbic acid was limited in its effect as compared to potassium bromate, as Burian pointed out.

General Conclusions

l-Ascorbic acid added to bread as crystalline ascorbic acid or as grapefruit juice appears to be rapidly oxidized during mixing and baking operations.

The *l*-ascorbic acid content of baked bread to which *l*-ascorbic acid had been added as crystalline *l*-ascorbic acid or as grapefruit juice was

found to be negligible and similar to that of conventional-type white bread.

Addition of grapefruit juice to bread does not appear to be justifiable from the viewpoint of increasing the *l*-ascorbic acid content of the baked bread.

Freshly baked cake retained approximately one-half, and cake 24 to 48 hours after baking retained slightly more than one-fourth, of the *l*-ascorbic acid in the added grapefruit juice.

Research of previous investigators which indicates that *l*-ascorbic acid is a dough conditioner equivalent to bromate, on a weight-for-weight basis, is confirmed.

d-Ascorbic acid is ineffective as a dough conditioner.

It is not possible to use grapefruit juice (in quantities tried, 7.5% to 30% on the basis of flour) as a source of *l*-ascorbic acid as a dough conditioner because of the detrimental effect of its acidity on the volume and texture of the bread.

Although grapefruit juice is not of value for increasing the *l*-ascorbic acid content of bread, it may have some value as a dough conditioner when used in small quantities.

Literature Cited

Bessey, O. A.
1938 Vitamin C—methods of assay and dietary sources. J. Am. Med. Assoc. **111**: 1290–1298.

Borsook, H., Davenport, H. W., Jeffreys, C. E. P., and Warner, R. C.
1937 The oxidation of ascorbic acid and its reduction in vitro and in vivo. J. Biol. Chem. **117**: 237–279.

Burian, K.
1939 Chemical treatment of flours. Chem. Obzor. **14**: 73–76 and 90–95.

Cathcart, W. H.
1938 Bread improvers and vitamin C. Am. Bakers' Assoc. Monthly Bulletin **2**: 116–117.

Jørgensen, H.
1935 Ein Beitrag zur Beleuchtung der hemmenden Wirkung von Oxydationsmitteln auf proteolytische Enzymtätigkeit: Über die Natur der Einwirkung von Kaliumbromat und analogen Stoffen auf die Backfähigkeit des Weizenmehles. I. Biochem. Z. **280**: 1–37 (abridgment in Mühlenlab. **5**: 113–126).
1939 Further investigations into the nature of the action of bromates and ascorbic acid on the baking strength of wheat flour. Cereal Chem. **16**: 51–60.

Melville, J., and Shattock, H. T.
1938 The action of ascorbic acid as a bread improver. Cereal Chem. **15**: 201–205.

Zavyalov, H., and Zavyalov, E.
1936 Enriching bakery products and pastry in vitamin C. Sovet. Mukomol'e Khlebopechemie **2**: (No. 9) 44–46. Through Chem. Abs. **32**: 3037, 1938.

THE RELIABILITY OF AN AUTOMATIC KERNEL COUNTER FOR 1,000-KERNEL-WEIGHT DETERMINATIONS

H. B. DIXON and S. R. SNIDER [1]

U. S. Department of Agriculture, Washington, D. C.

(Read at the Annual Meeting, May 1940)

Investigations by the Agricultural Marketing Service of the malting quality of barleys have shown a need for an automatic device to count barley or malt kernels for 1,000-kernel-weight determinations. A simple automatic apparatus that can be used in this determination to give consistent and reasonably accurate results will be of great service to malting and brewing laboratories.

The Lefco Automatic Kernel Counter shown in Figure 1, designed

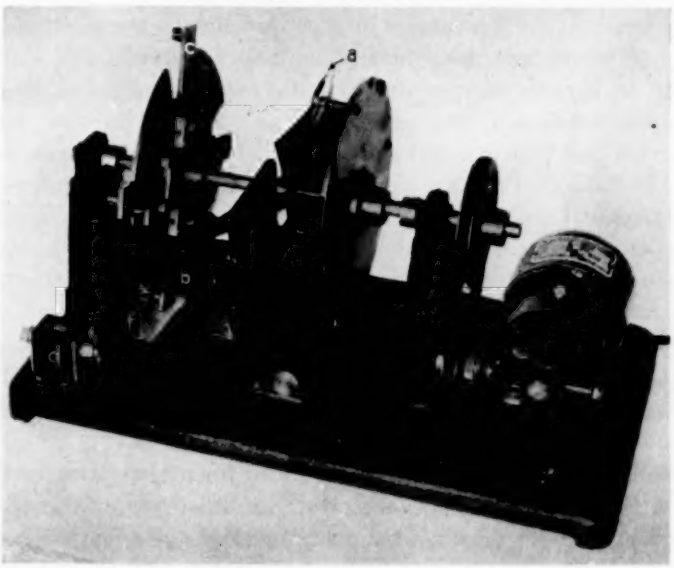

Fig. 1. Lefco Automatic Kernel Counter.—*a*, fingers for picking up one kernel at a time; *b*, stationary cam block with kernel notch and radial grooves; *c*, lever arm with wedge fins to move kernel out of notch and a cam knob to actuate counter arm; *d*, counter with automatic cut-off switch; *e*, box (in outline) for counted kernels; *f*, pan for discarded kernels.

for barley and malts and operating on mechanical principles only, has been tested in the course of our investigations. The operation of this device consists of picking up one kernel at a time from a hopper by radially moving fingers and then counting each kernel into a receptacle by means of cam and lever actions.

The fingers are designed to eliminate broken kernels and other seeds.

[1] Assistant Grain Technologist, Agricultural Marketing Service and Assistant Chemist, Bureau of Plant Industry, respectively.

Each kernel acts as an actuating cam as it is moved along the face of the stationary cam block by means of a radially moving lever arm. The cam block has radially cut grooves and a special notch to place each kernel in a vertical position. The lever arm is held in place by spring action and carries wedge fins that pass through the grooves in the cam block to move each kernel on the face of the block. The lever arm also carries a knob on the back as a cam to activate the arm of the counting mechanism as the moving kernel forces the lever arm away from the stationary block. Should two kernels be in the notch at the same time, the top kernel will force the lever arm away from the face of the block far enough to discard the bottom kernel into a pan. The driving motor of the device is automatically cut off at the indicated count of 500 and 1,000 on the Veeder intermittent counter.

The accuracy of 1,000-kernel-weight values obtained by use of an automatic counter depends primarily upon the ability of the counter to be nonselective of kernel size in accordance with the work of Shands (1937) [2] on the standard method of analysis. The effect of inaccurate counting of kernels on the weight values will increase as the 1,000-kernel-weight values increase. The importance of the time required to make the automatic count depends upon the number of determinations to be made. Preliminary investigations with the counter showed that duplicate 1,000-kernel-weight determinations on the basis of two 500-kernel counts for each determination were in remarkably close agreement. This close agreement was maintained even when the variations between duplicate 500-kernel determinations were slightly greater than the one-gram allowable error of the official hand-counting method. This work also indicated that samples of approximately 60 g. for eastern barleys and approximately 75 g. for western barleys were sufficient for the determination.

A study of the reliability of the counter for making the determination has been made by comparison of results so obtained with those obtained by the official hand-counting method. Barley samples having a large range in 1,000-kernel weight and samples having a large range in kernel sizes within the samples were used.

Samples and Methods

For the purpose of this study 10 eastern barley samples consisting of 9 six-row and 1 two-row samples, and 10 western barley samples consisting of 8 six-row and 2 two-row samples were selected on the basis of a wide range in test weight per bushel. Dockage was removed from all the samples, after which they were mixed thoroughly

[2] H. L. Shands, Barley and malt studies: III—the determination of kernel weight, Cereal Chem. 14: 532–539, 1937.

and divided into two portions by means of the Boerner Divider. One set of the eastern-barley portions was given odd numbers from 1 to 19 and reserved for analysis. The corresponding portions were given even numbers from 2 to 20 and were analyzed for percentages of various kernel sizes by means of a new experimental drum sizer. The percentages of thin kernels passing through the drum sieve with slots 0.0761-inch wide were discarded, thus making samples 2 to 20 representative of malt-house barleys. Hereafter samples from which thin kernels have been removed will be designated "thinless." The data on kernel-size separations of the thinless samples are shown in Table I.

TABLE I

DISTRIBUTION OF KERNEL SIZES IN THE EASTERN BARLEYS BY WEIGHT PERCENTAGES

Sample No.	Total sound kernels	Thin kernels	Size determinations after removing thin kernels					
			Total sound over 0.0761 in.[1]	Through 0.0859 in.[1]	Through 0.0937 in.[1]	Through 0.1015 in.[1]	Through 0.1093 in.[1]	Over 0.1093 in.[1]
	%	%	%	%	%	%	%	%
2	96.7	3.9	98.0	14.6	22.9	34.4	21.2	6.9
4	97.0	2.2	97.6	11.9	18.4	35.5	25.6	8.6
6	92.3	19.7	94.2	37.1	28.0	23.0	10.3	1.6
8	95.0	7.0	96.4	19.0	23.2	31.7	19.0	7.1
10	95.5	8.8	96.6	23.4	26.0	30.9	15.6	3.9
12	90.8	58.0	96.4	63.0	18.5	12.2	5.0	1.3
14	88.4	12.9	95.0	25.7	25.6	28.4	15.8	4.5
16	96.3	8.3	97.6	22.7	25.9	31.1	16.0	4.3
18	91.3	2.0	94.1	8.6	16.1	34.7	29.0	11.6
20	93.7	2.8	93.8	4.9	5.9	17.5	36.7	35.0

[1] Sieve dimensions correspond to 4¼, 5¼, 6, 6¼, and 7 sixty-fourths of an inch respectively, as used in barley graders.

The data show that the original samples had a range from 3.0% to 11.6% of material other than whole and sound barley kernels. This range was reduced to values from 2.0% to 6.2% by removal of the thin kernels. The samples are shown to have considerable variation in kernel size both between the samples and within each sample. Sample No. 12 is the only sample in which more than 50% of kernel weight occurred in one size classification. Obviously, the original samples containing thin kernels would have a much greater range in kernel sizes.

The western barley samples were not analyzed for kernel sizes. Odd numbers from 21 to 39 were assigned to one set of the portions, which were analyzed for 1,000-kernel weight by both methods. After making the tests the thin kernels were removed by means of the 0.0859-inch drum sieve, simulating malt-house procedure. The percentages

of thin kernels ranged from 3.2% to 44.1% in the original samples. The "thinless" samples were given the corresponding even numbers from 22 to 40. The two thinless samples later found to have the lowest and highest values for 1,000-kernel weight were sieved with the 0.1093-inch drum sieve to determine the percentages of extremely large kernels. The respective values were 19.0% and 62.1% for kernels failing to pass through this sieve.

The samples described above afford enough variation in 1,000-kernel weight, kernel size, and other factors to make a fair test of the reliability of the counter. In order to determine whether or not the counter action is affected by the size of the kernels, two series of 60-g. samples were prepared for analysis from the size separations of the eastern barleys. The first series consisted of eight samples having definite ratios by weight of different kernel sizes. Three samples contained two kernel-size limitations and five samples contained three size limitations. The 1,000-kernel-weight values were determined by making two 500-kernel counts with the counter and by hand counting whole barley kernels in the entire sample. The ratios of kernel sizes in the samples used and results of the two determinations are shown in Table II.

TABLE II

COMPARISON OF 1,000-KERNEL-WEIGHT DETERMINATIONS BY COUNTER AND BY HAND COUNT OF TOTAL SAMPLE ON EASTERN BARLEYS WITH DEFINITE WEIGHT RATIOS OF KERNEL SIZES

Sample No.	Weight ratio of kernel sizes in each sample as limited by sieve widths of —					1,000 kernel weight		Differences	
	0.0761 to 0.0859 in.[1]	0.0859 to 0.0937 in.[1]	0.0937 to 0.1015 in.[1]	0.1015 to 0.1093 in.[1]	Over 0.1093 in.[1]	Av. of duplicate counter tests	Hand count all barley in total sample	Between duplicate counter tests	Av. counter test minus hand count
						g.	g.	g.	g.
A	3	1	—	—	—	23.1	22.6	0.6	+0.5
B	1	1	—	—	—	25.6	25.4	0.3	+0.2
C	1	3	—	—	—	27.4	27.4	0.0	0.0
D	—	—	4	1	1	34.3	34.4	0.2	−0.1
E	—	—	3	2	1	34.4	34.5	0.4	−0.1
F	—	—	1	1	1	37.2	37.3	0.0	−0.1
G	—	—	1	4	1	38.0	38.0	0.3	0.0
H	—	—	1	2	3	40.6	41.2	0.8	−0.6

[1] Sieve dimensions correspond to 4½, 5½, 6, 6½ and 7 sixty-fourths of an inch respectively, as used in barley graders.

The variation in 1,000-kernel-weight values between duplicate 500-kernel tests as determined by the counter ranged from 0.0 g. to 0.8 g. The largest variations were for the samples having one of the kernel-size separations in the greatest proportion. The counter values

differed from the true values, obtained by hand counting the entire sample, by +0.5 g. for the lowest kernel-weight sample to −0.6 g. for the highest kernel-weight sample. The greatest differences occur with the samples having the largest proportion of kernels by weight and number within one-size separations of very small and very large kernels. For the entire range of 1,000-kernel-weight values the variations of the counter values from the hand values are well within the error allowed by the standard method.

The second series consisted of five samples having only kernels of one-size separation. Counter determinations were made as in the case of the first series of samples. The determination by the official method was used as the true value for making the comparisons. The whole barley kernels counted by the counter were checked and any other seeds present in the counted portion noted. The data on this series are shown in Table III.

TABLE III

COMPARISON OF 1,000-KERNEL-WEIGHT DETERMINATIONS BY COUNTER AND BY HAND ON EASTERN BARLEY SAMPLES HAVING ALL KERNELS LIMITED IN SIZE

Sample No.	Range in width of kernel [1]	No. kernels [2]	Weight		Actual composition of portion counted by mechanical counter			Differences		
								Between duplicate tests		Av. counter test minus av. hand test
			Counter	Hand	Barley	Oats	Other seeds, etc.	Counter	Hand	
	In.		*g.*	*g.*	*No.*	*No.*	*No.*	*g.*	*g.*	*g.*
I	0.0761–0.0859	500	10.7	10.7	514	4	1	—	—	—
		500	10.8	10.8	495	4	0	0.2	0.2	—
		1,000	21.5	21.5	1,009	8	1	—	—	0.0
J	0.0859–0.0937	500	14.6	14.5	501	2	0	—	—	—
		500	14.6	14.5	500	3	0	0.0	0.0	—
		1,000	29.2	29.0	1,001	5	0	—	—	+.2
K	0.0937–0.1015	500	16.9	17.1	500	0	0	—	—	—
		500	17.0	17.0	502	0	0	0.2	0.2	—
		1,000	33.9	34.1	1,002	0	0	—	—	−.2
L	0.1015–0.1093	500	19.4	19.2	498	0	0	—	—	—
		500	19.6	19.4	501	0	0	0.4	0.4	—
		1,000	39.0	38.6	999	0	0	—	—	+.4
M	Over 0.1093	500	24.6	24.7	500	0	0	—	—	—
		500	24.8	24.7	500	0	0	0.4	0.0	—
		1,000	49.4	49.4	1,000	0	0	—	—	0.0

[1] Sieve dimensions correspond to 4¼, 5¼, 6, 6¼, and 7 sixty-fourths of an inch respectively, as used in barley graders.

[2] In the case of the counter this refers to the registered number of kernels. In each case the 1,000-kernel portion is the two 500-kernel portions combined.

The range of 1,000-kernel-weight values is from 21.5 g. to 49.4 g., which is greater than the range for the first series of samples. These data indicate a high degree of accuracy for the kernel count. The counter failed to indicate nine barley kernels, eight oat kernels, and one other seed for sample No. 1. This, however, had very little effect on the weight value as indicated by the perfect check with the standard-method value. The variations in 1,000-kernel-weight values by the counter method from the values by the hand method are of the same order as those between duplicate tests by both methods and do not show a tendency to vary with the kernel-weight values. The comparisons indicate that for samples with all the kernels limited to a small range in size the counter can give reliable results for a large range in 1,000-kernel weight.

The 20 samples of eastern barleys previously described were analyzed by the counter method and the time required for the count on the 10 samples having no thin kernels was recorded in each instance. The number of kernels passing through the counter was checked for each sample. The entire test portions were returned to the samples before analyzing by the standard method. The time required for quartering and hand counting each of the 10 thinless samples was recorded. The comparative data on all the samples are shown in Table IV.

The 1,000-kernel-weight values ranged from 17.8 to 41.9 grams for these samples. The average variations between values for duplicate 500-count tests are 0.39 g. and 0.24 g. for the counter and standard methods, respectively. The differences between the 1,000-kernel-weight values by the two methods are not greater than 1 gram, the counter values ranging from 1.0 g. higher to 0.7 g. lower than the hand values. The extreme differences occur, as would be expected, with original samples containing thin kernels. The counter values average 0.26 g. greater than the hand values for the entire group of samples, but for the thinless samples the average difference is only -0.01 g. and the range is from $+0.6$ g. to -0.5 g.

The differences cannot be attributed to any inaccuracy of the kernel count because positive and negative differences occur in cases where the counter included more as well as when it included less than the recorded 1,000 kernels. The count on the thinless samples is very accurate considering that removal of thin kernels did not remove all broken kernels and other seeds from the samples. The agreement between the values for 1,000-kernel weight for the two methods is good for all the samples and particularly so for the thinless samples. These comparisons have been made without taking into account any

TABLE IV

TESTING THE KERNEL COUNTER WITH EASTERN BARLEYS

Sample No.	Type of barley, 2- or 6-row	1,000 ker. wt., av. duplicate tests by		Differences in 1,000-kernel-weight values			Hand check on automatic count of 1,000 kernels	Counting time in minutes and seconds		
				Duplicate tests		Av. counter minus av. hand		Counter	Hand [1]	Diff., counter minus hand
		Counter	Hand	Counter	Hand					
		g.	g.	g.	g.	g.				
1 [2]	6	30.1	29.1	0.2	0.2	+1.0	1,005	—	—	—
2 [3]	6	30.7	30.5	0.2	0.2	+0.2	994	10:35	9:30	+1:05
3	6	33.1	33.0	0.6	0.0	+0.1	1,005	—	—	—
4	6	32.7	33.2	0.6	0.0	−0.5	1,007	14:20	11:57	+2:23
5	6	25.6	24.9	0.0	0.6	+0.7	975	—	—	—
6	6	29.0	28.8	0.0	0.4	+0.2	992	13:50	12:30	+1:20
7	6	29.0	28.7	0.8	0.6	+0.3	995	—	—	—
8	6	29.8	30.2	0.4	0.4	−0.4	997	13:00	11:10	+1:50
9	6	28.3	28.2	0.6	0.0	+0.1	1,004	—	—	—
10	6	30.3	29.7	0.2	0.2	+0.6	1,002	13:00	11:45	+1:15
11	6	18.1	17.8	0.2	0.4	+0.3	1,028	—	—	—
12	6	24.1	24.0	0.6	0.0	+0.1	991	14:15	11:10	+3:05
13	6	28.1	27.4	0.6	0.4	+0.7	947	—	—	—
14	6	29.4	29.5	0.4	0.2	−0.1	985	13:05	11:55	+1:10
15	6	28.4	27.4	0.4	0.0	+1.0	1,000	—	—	—
16	6	29.7	29.7	0.2	0.2	0.0	995	13:10	10:10	+3:00
17	6	33.7	32.9	0.6	0.6	+0.8	1,001	—	—	—
18	6	34.0	33.7	0.0	0.2	+0.3	1,000	13:10	10:45	+2:25
19	2	41.4	41.0	0.4	0.0	+0.4	985	—	—	—
20	2	41.4	41.9	0.8	0.2	−0.5	1,001	14:30	10:10	+4:20
			Av.	0.39	0.24	+0.26			Av.	+2:11

[1] Includes quartering time.
[2] Counter sample weighed from can.
[3] Both samples by quartering.

possible differences between the test portions due to sampling procedure.

The counter required an average of 2 minutes and 11 seconds more time to count two 500-kernel portions than was required by the hand method of quartering and counting the same number of kernels. The time comparisons were not made for continuous operations but on the basis of a single determination. It is impossible for an analyst to maintain the same speed for a large number of samples.

The samples of western barleys were used to furnish data similar to that determined on the eastern barleys with four exceptions in the procedure. The exceptions are: (1) The thinless samples were prepared from the portions tested as original samples. (2) For both methods the test portions of the thinless samples were selected by a Boerner Divider. (3) The total number of kernels was determined on the test portions used for counter tests on the original samples, and on the test portions used for both counter and hand tests on the

thinless samples. (4) The time data and number of whole kernels counted by the counter were recorded for the determinations on the 10 original samples. The data for the comparison of the counter method and official method are shown in Table V.

TABLE V

TESTING THE KERNEL COUNTER WITH WESTERN BARLEYS

Sample No.	Type of barley, 2- or 6-row	1,000 ker. wt., av. duplicate tests by		Differences in 1,000-kernel-weight values			Hand check on automatic count of 1,000 kernels	Counting time in minutes and seconds		
				Duplicate tests		Av. counter minus av. hand		Counter	Hand [1]	Diff., counter minus hand
		Counter	Hand	Counter	Hand					
		g.	g.	g.	g.	g.				
21 [2]	6	34.3	34.9	0.2	0.6	−0.6	994	21:57	15:00	+6:57
22 [3]	6	39.5	39.9	0.2	0.8	−0.4	—	—	—	—
23	6	38.7	39.5	0.9	1.0	−0.8	991	17:20	13:30	+3:50
24	6	43.0	44.4	0.0	0.7	−1.4	—	—	—	—
25	6	31.4	32.3	0.7	0.9	−0.9	986	17:30	13:00	+4:30
26	6	37.3	38.1	0.2	0.0	−0.8	—	—	—	—
27	6	49.2	49.7	1.1	1.0	−0.5	975	18:20	15:00	+3:20
28	6	49.4	50.9	1.0	0.0	−1.5	—	—	—	—
29	6	39.2	39.8	1.1	0.8	−0.6	994	16:28	11:40	+4:80
30	6	42.9	44.4	0.2	1.3	−1.5	—	—	—	—
31	6	47.1	46.9	0.9	1.0	+0.2	989	17:40	13:25	+4:15
32	6	47.4	47.9	0.3	0.7	−0.5	—	—	—	—
33	6	42.2	42.9	1.4	0.2	−0.7	978	14:58	12:35	+2:53
34	6	42.8	43.5	0.3	0.6	−0.7	—	—	—	—
35	6	40.5	41.1	0.8	1.0	−0.6	962	14:10	15:00	−0:50
36	6	41.8	42.4	0.1	0.3	−0.6	—	—	—	—
37	2	40.2	40.0	0.2	0.0	+0.2	985	13:10	14:30	−1:20
38	2	41.2	41.2	0.8	0.1	0.0	—	—	—	—
39	2	38.2	39.0	0.5	0.0	−0.8	992	13:25	14:00	−0:35
40	2	39.3	39.5	0.1	0.8	−0.2	—	—	—	—
			Av.	0.55	0.59	−0.66			Av.	+2:44

[1] Includes quartering time.
[2] Counter sample weighed from can.
[3] Both samples of minimum size from Boerner Divider.

The 1,000-kernel-weight values by the hand method show a range from 32.3 g. to 50.9 g. Removal of thin kernels increased the weight value as much as 5.8 g. for samples No. 25 and No. 26 where 44.1% of the original sample was thin kernels. The differences between duplicate tests are on the average 0.55 g. and 0.59 g. for the counter and hand methods, respectively. Differences equal to or greater than the allowable error of one gram occur in four instances for the counter method and in five instances for the hand method. Apparently close checks between duplicates are more difficult to obtain with this class of barleys.

The counter values average 0.66 g. lower than the hand values. Only two original samples show the counter values to be higher than

hand values. In the case of Nos. 24, 28, and 30, all thinless samples, the counter values are approximately 1.5 g. lower than the hand values; but two of these samples show a greater difference between duplicate hand tests than that shown for duplicate counter tests. However, all three of these samples were long-awned varieties. The check on the number of kernels in the automatic count on the original samples shows that only in three instances could the error in count be considered as the only factor causing the low values by the counter method. The time required by the counter averaged 2 minutes and 44 seconds longer than the hand count, on the basis of a single determination.

The weight of actual number of kernels determined as described in the procedure for these kernel-weight values was used as a basis to determine the average differences for each method and for each test sample. The data are shown in Table VI.

TABLE VI

COMPARISON OF THE 1,000-KERNEL-WEIGHT VALUES OF TABLE V WITH VALUES DETERMINED ON THE BASIS OF THE TOTAL NUMBER OF KERNELS IN THE SAMPLE

Sample No.	Automatic counter sample			Hand counted sample			Diff., total counter minus total hand
	1,000 ker. wt.		Diff., duplicates minus total sample	1,000 ker. wt.		Diff., duplicates minus total sample	
	Av. duplicate tests	Total kernels hand counted		Av. duplicate tests	Total kernels hand counted		
	g.	g.	g.	g.	g.	g.	g.
21	34.3	34.2	+0.1	34.9	—	—	−0.7
22	39.5	39.9	−0.4	39.9	39.9	0.0	0.0
23	38.7	39.3	−0.6	39.5	—	—	−0.2
24	43.0	43.4	−0.4	44.4	44.1	+0.3	−0.7
25	31.4	31.4	0.0	32.3	—	—	−0.9
26	37.3	37.4	−0.1	38.1	37.5	+0.6	−0.1
27	49.2	50.3	−1.1	49.7	—	—	+0.6
28	49.4	50.5	−1.1	50.9	50.4	+0.5	+0.1
29	39.2	39.6	−0.4	39.8	—	—	−0.2
30	42.9	44.1	−1.2	44.4	44.6	−0.2	−0.5
31	47.1	47.6	−0.5	46.9	—	—	+0.7
32	47.4	48.1	−0.7	47.9	47.6	+0.3	+0.5
33	42.2	42.9	−0.7	42.9	—	—	0.0
34	42.8	43.7	−0.9	43.5	43.5	0.0	+0.2
35	40.5	40.9	−0.4	41.1	—	—	−0.2
36	41.8	42.2	−0.4	42.4	42.0	+0.4	+0.2
37	40.2	40.3	−0.1	40.0	—	—	+0.3
38	41.2	41.3	−0.1	41.2	41.0	+0.2	+0.3
39	38.2	38.1	+0.1	39.0	—	—	−0.9
40	39.3	39.5	−0.2	39.5	39.5	0.0	0.0
		Av.[1]	−0.55		Av.[1]	+0.21	Av.[1] 0.0
		Av.[2]	−0.46				Av.[2] −0.075

[1] Only even numbered samples.
[2] All samples.

The total number of kernels in the hand-test samples ranged from 1,006 to 1,285 and in the counter test samples from 1,271 to 1,573.

The 1,000-kernel-weight values by the counter averaged 0.46 g. lower than the true values (based on the total number of kernels), for all of the samples. The average for the thinless samples is 0.55 g. lower. These average differences are influenced to a considerable extent by the large differences for long-awned samples, 27, 28, and 30. The range for the difference values for all samples is from +0.1 g. to −1.2 g., all but two of which are negative values.

The 1,000-kernel-weight values by the hand method on the thinless samples were 0.21 g. on the average higher than the true values. The difference values show the hand method to have a value lower than the true value in only one instance. The range in difference values was from +0.6 g. to −0.2 g. Since the comparison of the hand-method values to true values has been made on thinless samples only it is quite probable the average difference would have been even higher if the original samples had been included.

A comparison of the true 1,000-kernel-weight values for the counter samples and the hand samples shows that the true values of the counter samples averaged 0.075 g. lower than those of the hand samples, and that the range was from 0.9 g. lower to 0.7 g. higher.

Discussion of Results

The results from the samples in Tables II and III indicate that counter determinations are reliable for samples having considerable range in kernel sizes and in 1,000-kernel weight. The range of +0.5 g. to −0.6 g. deviation found with extreme conditions is in accordance with the deviation values shown by Shands (footnote 2). The comparative data in Table IV for samples of eastern barleys that represent dockage-free samples and malt-house samples show that counter determinations on the average may be expected to be 0.26 g. too high. This average and the range of variations shown indicate that the counter values can be relied upon to have approximately the same significance as the standard-method values. This is particularly true of the values for the malt-house samples.

The data on the western barley samples for the two methods show that the counter values on the average are 0.66 g. too low. The consistency of low values is significant.

However, the larger average of 0.66 g. and smaller range in the variations than shown by the data for the eastern barley samples do not indicate a material difference in the degree of accuracy for the counter determinations. The low values can perhaps be attributed to the increasing deviation in single-kernel weight for increasing size of

kernel, and the fact that an oat or similar material in these samples that was counted as barley, weighs considerably less than a barley kernel.

The data on the same samples in Table VI are based on the actual weight of the kernels in the two determinations and confirm the underestimation of 1,000-kernel weight by the counter method. The average error is reduced, however, from 0.66 g. to 0.46 g. On the other hand, the data show that the standard hand method has overestimated the 1,000-kernel weight on the average by 0.21 g. Two analysts making these determinations independently had a tendency to select larger than average kernels. This may be the case with most analysts with this class of barley.

The average difference between the samples based on the weight of all the kernels is 0.075 g. Therefore, taking into account either one or both of the values of 0.21-g. average overestimation by the hand method and the 0.075-g. average difference between the samples, we have a reduction of the 0.66-g. average underestimation by the counter determinations as shown by the comparison of methods in Table V.

It is quite probable that determinations on the basis of total number of kernels for the eastern barley samples would have shown low values for the official method and considerable difference between the samples tested by both methods. This assumption can be based on the fact that the eastern samples have a large proportion of small-size kernels and that the test portions were not mechanically selected.

Conclusions

The investigations on the reliability of the Lefco Automatic Counter for the determination of 1,000-kernel weight of barleys herein reported support the following conclusions:

1. Counter determinations on samples having all kernels within one small range of kernel size are equally reliable over a large range in 1,000-kernel weight.
2. A mixture of kernel sizes within a sample affect the counter determinations only when there is a large proportion of an extreme kernel size in the sample.
3. Counter determinations on dockage-free eastern barleys are consistently higher than official-method determinations over a large range of kernel-weight values.
4. Counter determinations on samples of eastern barley without thin kernels consistently check the official method determinations within the standard deviation of the official method.
5. Counter determinations on dockage-free samples and samples

without thin kernels of Western barleys are consistently lower than official-method determinations.

6. The variation of counter determinations from official-method determinations mentioned in conclusions 2, 3, and 5 are within the standard deviations of the official method.

7. The comparison of 1,000-kernel-weight values on the basis of all the kernels in the test portions for the western barley samples indicates that if smaller test portions had been used for the counter determinations on the eastern barleys and if these portions had been mechanically selected, even more accurate results would have been obtained.

8. The use of the automatic counter for 1,000-kernel-weight determinations removes any personal error and can maintain an efficiency for a number of determinations that it is impossible to attain by the official method.

TESTING THREE FLOURS IN FIVE CAKE FORMULAS

DONALD WADE and F. J. COUGHLIN

The Procter & Gamble Company, Ivorydale, Ohio

(Read at the Annual Meeting, May 1940)

The purpose of the cake-baking test is to differentiate flours. Ability to distinguish among flours should, of course, be reproducible in different laboratories. Three flours were selected for this work, mainly because of their differences in characteristics. Pup loaves of bread were made, and the tentative A. A. C. C. cake formula and four other cake formulas were used. The objects of this work were: (1) to correlate cake quality with flour analyses, and (2) to determine the type of cake formula which would measure differences among flours most satisfactorily.

Flour Characteristics

The three flours selected are referred to as *X*, *Y*, and *Z*. The analyses of these flours are shown in Table I.

X and *Y* were commercial cake flours widely known in the baking industry as excellent cake flours. The main difference between the two is the lower viscosity of *Y*. Flour *Z* was purposely selected because of its analysis. The high ash, protein, and pH, its viscosity characteristics, and coarse granulation indicate a poor flour for cake.

Figure 1 shows test loaves of bread baked from the three flours each

TABLE I

FLOUR ANALYSES

Flour	H_2O	Ash (15% m.b.)	Protein (15% m.b.)	pH	Viscosity, cc. lactic acid					20 min. granulation				(30 min.) Thru 250
					0	1	3	5	7	On 165	On 230	On 250	Thru 250	
X	11.65	0.339	7.68	5.13	4	30	38	42	42	—	1.0	41.0	58.0	83.0
Y	10.65	0.358	7.74	4.90	5	12	17	19	19	—	—	46.0	54.0	80.0
Z	12.20	0.501	8.89	5.52	15	13	36	45	46	—	6.0	54.0	40.0	62.0

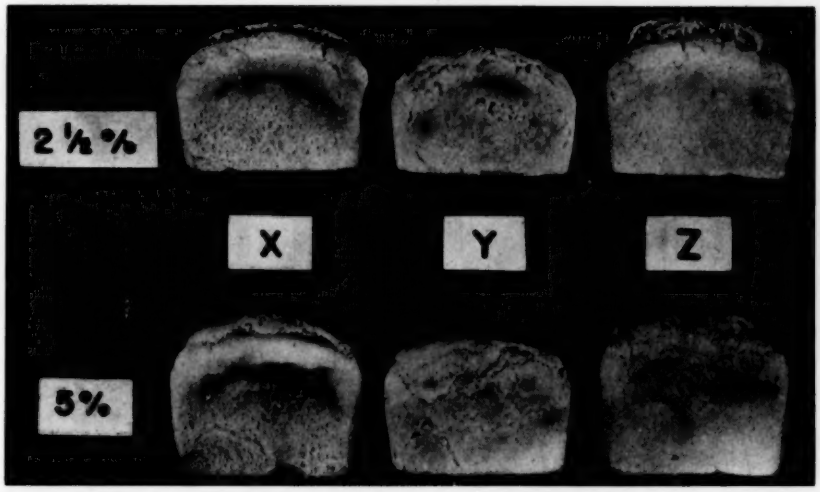

Fig. 1.. Loaves baked with 2½% and 5% of sugar.

at two sugar concentrations, 2½% in the upper row and 5% in the lower row. As might be expected, flour Z gave the largest loaf.

Cake Baking Tests

The five cake formulas used with the three flours are shown in Table II.

TABLE II

FIVE CAKE FORMULAS USED

Formula	Description	Flour	Sugar	Shortening	Salt	Baking powder	Eggs	Milk
OT	Present tentative	100	96	25	1.5	4.0	32	88
OM	Modified tentative	100	96	35	1.5	4.0	35	88
P	Rich pound cake	100	120	70	3.8	0.0	70	50
YL	Rich yellow layer	100	140	55	3.8	6.3	60	105
WL	Rich white layer	100	140	55	3.8	6.3	75	95

Formula *OT* is the present tentative test method. *OM* is a modified formula with slightly increased shortening and eggs and was one of the formulas used by the 1939–40 committee. *P* is a rich commercial yellow pound cake, high in sugar, shortening, and eggs. *YL* is a rich commercial yellow layer cake. *WL* is a rich white layer formula almost identical with a formula used by the 1938–39 committee. Because of the nature of the formulas, special cake shortening was used in each of the last three.

Figure 2 shows cakes made according to the present tentative test method. The volumes of the cakes were almost identical, and the symmetry was good in every case. The second cake had the best grain,

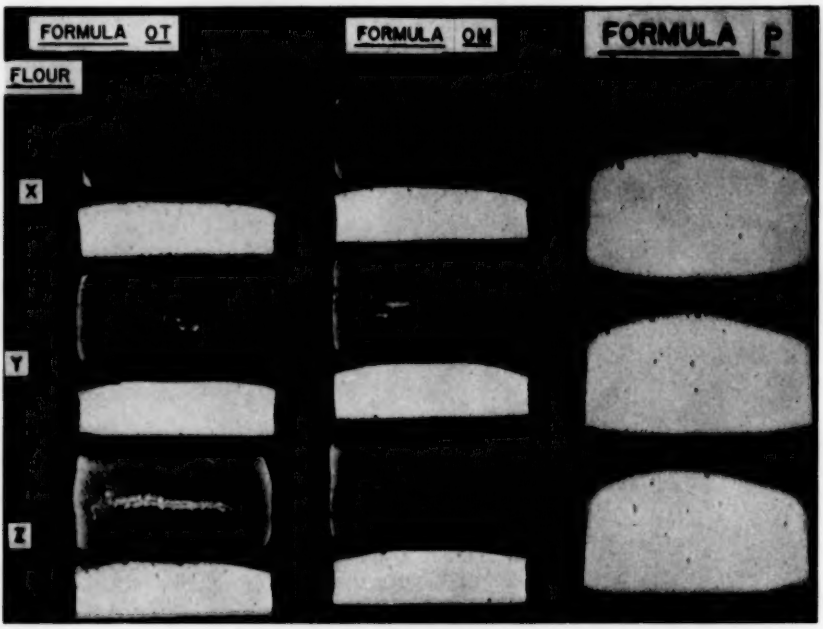

Fig. 2. Present tentative method.

Fig. 3. Modified formula with shortening and eggs slightly increased in comparison with formula *OT*.

Fig. 4. Rich yellow pound cake formula.

but all three would be judged satisfactory. Using the standard score sheet, *Y* showed the best score with 86, with *X* and *Z* practically equal at 81 and 80.

In Figure 3, showing formula *OM* which is not very different from the previous formula, the results are practically the same as in the case of formula *OT*. *Y* is best with *X* and *Z* tied for second and third. The actual scores were 81 for *X*, 88 for *Y*, 82 for *Z*.

Figure 4 shows the three flours used in the rich yellow pound cake formula. With this formula, all three flours gave almost the same cake. That is, this cake formula did not distinguish any differences among the three flours. The actual scores of the cakes were 91, 90, and 92.

Figure 5 shows rich white layers made from the three flours. In this formula, flours *X* and *Y* gave cakes better in volume, grain, silkiness and color than flour *Z*. The scores of the cakes were 88, 90, and 83—in close agreement with what are generally considered as required analytical characteristics.

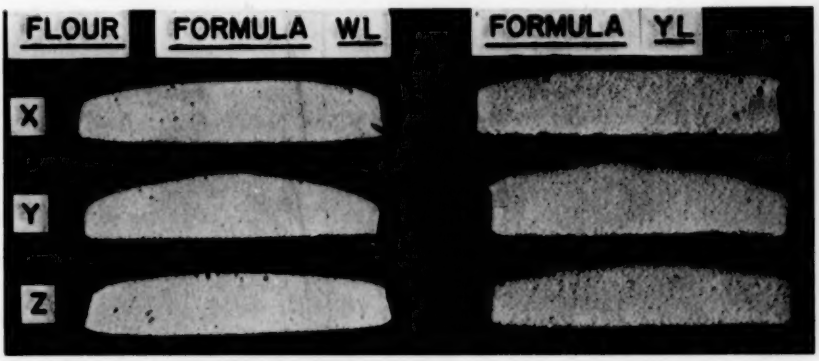

Fig. 5. Rich white layer cake formula.

Fig. 6. Rich yellow layer cake formula.

Figure 6 shows the rich yellow layer cakes. The volume of the cake made from flour *Z* is distinctly poorer than the other two but in other characteristics the cakes are practically equal.

Scoring

The scores of these cakes cannot be compared *between* formulas. Even though all of the scores of the 15 cakes fall in a fairly close range, there is a wide difference in their quality as judged from the bakers' and consumers' points of view. This is especially true if the formulas are compared from the standpoint of eating quality. If only one cake formula is considered, eating qualities are important only when the flour produces differences in eating quality. On the other hand, when various cake formulas are considered, as in this work, the official scoring system is not flexible enough to show these large differences in eating quality. Standards generally accepted for scores on the official cake also do not allow these differences to be shown.

Conclusion

Based on this study, it is indicated that:

1. Flours which are decidedly inferior in one cake formula may be entirely satisfactory in another. Relative ranks by one formula may be markedly changed if another cake formula is used.

2. Analytical data ordinarily obtained are not sufficient to predict the suitability of a cake flour for a variety of cake formulas.

Acknowledgments

We wish to express our thanks to H. M. Simmons for supplying one of the flour samples, to W. H. Hanson for the granulation data, to H. J. Loving of the Kroger Food Foundation for the miniature bread bakings and some of the analyses, and especially to George Garnatz for his suggestions and cooperation during the work.

THE EFFECT OF SHORTENING ON THE A. A. C. C. BREAD-BAKING TEST

J. A. SHELLENBERGER

The Mennel Milling Company, Toledo, Ohio

(Read at the Annual Meeting, May 1940)

Shortening has not been included among the ingredients used in the A. A. C. C. baking test formula for bread, although it is a common constituent of commercial bake-shop formulas. A perusal of the literature pertaining to experimental baking reveals that, even though lacking official sanction, a great many of the baking results reported in connection with various phases of cereal chemistry have been made with formulas which included shortening. Indeed it is surprising that the use of shortening in the experimental baking test has not become even more prevalent than it is since various treatises and books on baking exalt its usefulness and claim such virtues as improved loaf volume, better appearance, softer crumb, and more silky texture when shortening is incorporated in the dough.

One of the obvious reasons for omitting fats from the experimental baking test has been the desire to avoid the introduction of another variable. It is generally recognized that there is considerable difference in the shortening power of various types of fat. Heald (1937) reported the differences in loaf volume resulting from the use of equivalent quantities of animal, vegetable, and compounded shortening in a bread formula. However, there appears to be no information available which precisely indicates the variation in loaf volume and bread characteristics

which can be expected to result when various types of commercial shortening are included in the basic A. A. C. C. baking formula.

Review of Previous Work

Shortening as an ingredient of the basic A. A. C. C. baking test formula has attracted some attention. Merritt, Blish, and Sandstedt (1932) reported that shortening added to the formula increased loaf volume. As indicated previously, Heald (1937) reported the effect of different animal, vegetable, and compounded fats on loaf volume, grain, and texture when the quantity of shortening in each case varied over a range from zero to 12%. It was found that the maximum reaction was obtained when the dough contained 4% shortening and all classes of fat caused an increase in loaf volume and better internal loaf characteristics.

The effect of shortening on the gas production and retention of doughs has also been studied by Heald (1939). He found that the rate of gas production was retarded by the inclusion of shortening in the formula. Gas retention, however, was found to be influenced by the flour grade and bleach as well as by the inclusion of shortening; therefore, no general rule can be formulated which will hold for all cases.

Heald (1939) recommends that shortening be included in the A. A. C. C. baking formula; and Sandstedt and Blish (1939), following their study of the influence of sugar levels on the baking value of commercial flours, make a similar recommendation.

From the review of past accomplishments it appeared that additional information should be obtained before the inclusion of shortening in the basic baking-test formula could be safely recommended. Information concerning the influence of different types of fat must of necessity be available, since obviously, if the shortenings regularly purchasable on the market produce a significant difference in baking results, then the problem of recommending shortening for the baking formula becomes complicated by the additional task of designating a specific type of fat.

It is unfortunate that more of the recent researches in physical chemistry are not immediately applicable to the study of dough. Platt and Fleming (1923), however, have discussed some of the surface phenomena involved in the use of shortening in a dough. The idea of molecular orientation developed by Hardy (1913) and demonstrated by Langmuir (1917) has thrown important light on many of the fundamental problems related to shortenings and lubricants. Because of the complexity and diversity of the surfaces in a bread-dough system and the known variation in the surface area covered per unit quantity of fat, depending upon such variables as the degree of unsaturation, number and kind of polar groups, and the properties of the surface, it is

difficult to present a truly adequate picture of the distribution of fat in a dough. For practical purposes the action of fat in a dough during mixing can be visualized as the internal lubricant. The fat spreads on the gluten and starch surfaces and coats them with a thin film of grease, thus separating the dough constituents and permitting greater ease of slippage between the different parts.

Experimental

The effects of shortening on dough development.—A micro recording mixer was used to study the effect of shortening on dough development. Salt, sugar, and yeast doughs, with and without the addition of shortening, were compared. Figure 1 shows typical curves. The upper curve indicates the mixing characteristics when no fat was included in the dough, and the lower curve shows the characteristics of a similar dough but including 3% of a hydrogenated shortening introduced into the mixing bowl at 80°F.

It is evident that the inclusion of fat in a dough lowers its consistency but does not materially alter the rate of development. There is a tendency for the break-down of the gel structure to be less drastic when fat is present as is illustrated in Figure 1. The two curves almost coincide

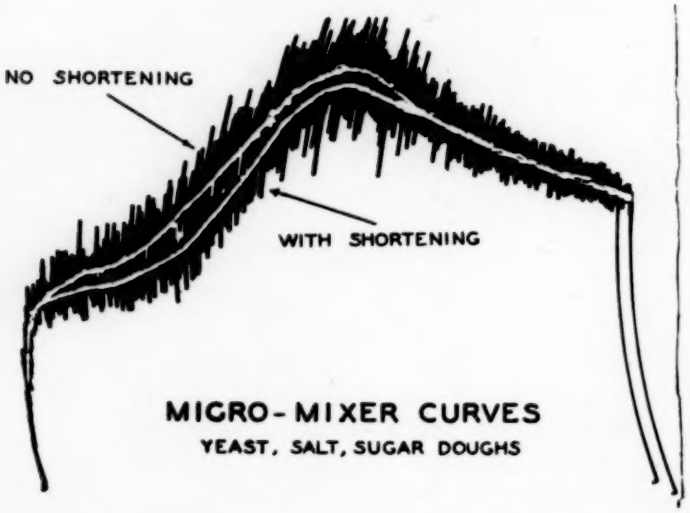

Fig. 1. The effect of 3% of shortening on dough properties as indicated by a micro recording mixer.

after a similar mixing period, although at the peak development point the nonfat curve has a higher consistency and therefore the steeper slope on the falling-off portion of the curve.

It seems apparent from a study of recording dough-mixer curves that the inclusion of shortening in the baking test would involve no readjustment insofar as mixing considerations are concerned. The validity of this statement is strengthened by the fact the National Micro Recording Dough Mixer has the same type of dough-developing action as the Swanson-Working (1936) mixer.

The effect of different shortenings on loaf volume and bread score. —Seven shortenings were obtained from common commercial sources. The samples represented typical vegetable, animal, and compounded fats, a price differential between samples within a given type, and one liquid shortening. Effort was made to have sufficient diversity of selection to include any type such as might be purchased for the average cereal laboratory. Following the recommendation of Heald (1939) 3% of each of these shortenings was included in the baking formula and baking tests conducted according to the method outlined in Cereal Laboratory Methods (1935).

Two different flour grades were used for the baking test. One was a baker's patent with the following analysis on a 15% moisture basis: protein 12.8%, ash 0.40%, absorption 63.0%, diastatic activity 310 mg. maltose. The other was a straight-grade family flour corresponding to the following description: protein 10.6%, ash 0.42%, absorption 60.3%, diastatic activity 234 mg. maltose. Each time the experiment was repeated duplicate doughs were baked using each shortening, and the entire series of fats was always included in one bake. The relative position of the doughs containing the different shortenings were progressively changed in respect to one another in the baking sequence in order to avoid errors which might occur if the doughs maintained fixed positions in the series. The entire shortening series was rebaked 20 times with the baker's flour and 10 times with the family flour.

Results

The results of this investigation are recorded in Tables I and II. Loaf volume and bread score were recorded as criteria of the change produced by different fats. The bread score was obtained by assigning numerical values to the important external and internal loaf characteristics, including the volume. The mean loaf-volume and bread-score values reported for the seven shortenings and the control is the average of 40 loaves in Table I and 20 loaves in Table II. It is evident that the inclusion of shortening in the baking formula produces bread of greater volume and higher numerical score. In these experiments there was no indication that shortening tended to reduce the variability of either the

TABLE I

BAKER'S GRADE FLOUR

Significance of Differences between the Mean Loaf Volumes and Bread Scores for Shortening Types and those Obtained by an Individual Shortening

Shortening type	Loaf Volume (cc.) Mean volume	Stand. deviation	Coef. of variation	Stand. error of mean	$\frac{D^*}{E_D}$
None	731.5	24.11	3.30	5.392	3.896
A	756.6	21.53	2.84	4.813	0.178
B	761.2	21.67	2.85	4.847	0.448
C	761.6	24.27	3.19	5.428	0.574
D	767.5	27.15	3.54	6.071	1.349
E	756.5	26.43	3.49	5.909	0.163
F	759.4	20.69	2.72	4.626	0.290
G	767.2	27.38	3.57	6.124	1.295
	Bread Score				
Shortening type	Mean score	Stand. deviation	Coef. of variation	Stand. error of mean	$\frac{D^*}{E_D}$
None	94.0	1.36	1.44	0.299	4.713
A	96.1	1.19	1.25	0.268	0.352
B	95.9	1.02	1.07	0.229	0.166
C	96.3	1.67	1.74	0.374	0.864
D	96.3	1.09	1.13	0.244	0.945
E	96.5	0.89	0.92	0.198	1.313
F	96.2	1.02	1.06	0.227	0.919
G	96.4	1.09	1.14	0.245	1.157

* D = Difference between means. $P = 0.05$
E_D = Standard error of difference between means. $N = 26$
Significant difference when $D/E_D > t$. $t = 2.056$

loaf volume or the bread score. In fact, the family-flour loaves baked without the addition of fat had the smallest coefficient of variation of loaf volume of the entire group.

Statistical analysis reveals a significant difference between the volumes of loaves obtained when shortening was included in the formula, compared to similar loaves baked from doughs without shortening. However, the different shortening types did not produce a significant difference. The bread scores followed this same rule and both flour types led to precisely the same result. In determining the probability that the difference between means is significant, use was made of Fisher's (1934) table of t values. A significant difference is one that gives a greater calculated value of t (see note at bottom of Tables I and II) than the corresponding reading from the table for $P = 0.05$.

Discussion

This investigation indicates that shortening could be included in the A. A. C. C. bread-baking formula without necessarily designating a specific type of fat. This statement should not be construed to mean

TABLE II

FAMILY GRADE FLOUR

Significance of Differences between the Mean Loaf Volumes and Bread Scores for Shortening Types and those Obtained by an Individual Shortening

	Loaf Volume (cc.)				
Shortening type	Mean volume	Stand. deviation	Coef. of variation	Stand. error of mean	$\frac{D^*}{E_D}$
None	630.8	12.57	1.99	3.975	4.919
A	657.7	33.17	5.04	10.490	0.235
B	661.7	23.28	3.52	7.361	0.156
C	669.2	26.40	3.94	8.349	1.047
D	664.3	15.94	2.40	5.040	0.579
E	661.1	20.57	3.11	6.504	0.009
F	668.0	18.32	2.74	5.793	1.037
G	670.3	19.32	2.88	6.111	1.266
	Bread Score				
Shortening type	Mean score	Stand. deviation	Coef. of variation	Stand. error of mean	$\frac{D^*}{E_D}$
None	89.3	1.32	1.47	0.465	2.887
A	91.6	1.51	1.65	0.522	0.776
B	91.1	0.98	1.07	0.830	0.107
C	91.7	1.67	1.83	0.579	0.935
D	91.4	1.58	1.73	0.547	0.457
E	91.8	0.98	1.06	0.335	1.174
F	91.2	1.00	1.10	0.348	0.218
G	90.7	0.79	0.87	0.275	0.578

* D = Difference between means. $P = 0.05$
E_D = Standard error of difference between means. $n = 16$
Significant difference when $D/E_D > t$. $t = 2.120$

that shortening can be used indiscriminately in the bread-baking test, regardless of composition, workability, consistency, or cost. It is essential to remember that the fats used in this experiment were incorporated in the doughs only at the relatively low level of 3% and no consideration was given to such important aspects as the keeping properties of these fats or their influence on taste, odor, or palatability of the bread. However, this experiment does indicate that at the 3% level there was no significant difference between the baking-test results obtained from the use of various types of high-grade shortening such as are readily obtainable on the market.

The literature is replete with references to the variability of animal and vegetable fats. In the interest of keeping conditions as constant as possible, it would be advisable to use an all-hydrogenated shortening, since these can be manufactured under rigid specifications, are widely distributed, and consequently available to practically every cereal chemist.

Summary

Shortening is extensively employed in test-bake formulas although it does not have the official sanction of the A. A. C. C. The suggestion that shortening be included among the ingredients in the basic bread-baking formula is not new, and the literature already contains references to the effect of fats on bread characteristics and on the gas production and gas retention of doughs. Also, information is available on the effect of different types of fats used at various levels on the properties of bread.

The present study represents an effort to supply additional information regarding the effects of the inclusion of fats on the A. A. C. C. basic bread-baking test. The influence of fats on the mechanical development of doughs had not been recorded and essential knowledge regarding the significance of differences in bread quality caused by the use of various types of commercial shortening seemed inadequate.

Shortening does not materially affect the rate of dough development as measured by the National Recording Micro Mixer; it does, however, reduce the detrimental effect of overmixing and decreases dough consistency.

Statistical analysis of the data obtained by rebaking a series of doughs varying only by the inclusion of 3% of seven different fats revealed that no significant variations occurred in either the loaf volumes or bread scores. A significant difference in bread characteristics was produced between the bakes containing shortening and those without fats. The inclusion of shortening in the baking-test formula will reduce the stickiness of doughs and produce bread of larger volume, better grain, and better texture.

It appears that shortening to the extent of approximately 3% can be safely recommended for inclusion in the A. A. C. C. basic baking-test formula without introducing a significant variable, even though no specified fat is mentioned. In the interest of uniformity, however, it would possibly be advisable to recommend an all-hydrogenated shortening.

Literature Cited

American Association of Cereal Chemists
1935 Cereal laboratory methods (3rd ed.), p. 75.

Fisher, R. A.
1934 Statistical methods for research workers (5th ed.). Oliver and Boyd, Edinburgh.

Hardy, W. B.
1913 The influence of chemical condition upon interfacial tension. Proc. Roy. Soc. (London) **88** (A) : 303–313.

Heald, W. L.
1937 The effect of different types of shortening on white pan bread. Cereal Chem. **14**: 481–488.

1939 Gas retention as affected by including shortening in the tentative A. A. C. C. basic test-bake formula. Cereal Chem. **16**: 817–820.

Langmuir, I.
1917 The constitution and fundamental properties of solids and liquids. J. Am. Chem. Soc. **39**: 1848–1906.

Merritt, P. P., Blish, M. J., and Sandstedt, R. M.
1932 Report of the activities of the A. A. C. C. baking research fellowship. Cereal Chem. **9**: 175–238.

Platt, W., and Fleming, R. S.
1923 The action of shortening in the light of the newer theories of surface phenomena. Ind. Eng. Chem. **15**: 390–394.

Sandstedt, R. M., and Blish, M. J.
1939 The question of sugar levels in laboratory baking. Cereal Chem. **16**: 36–42.

Swanson, C. O., and Working, E. B.
1926 Mechanical modification of dough to make it possible to bake bread with only the fermentation in the pan. Cereal Chem. **3**: 65–83.

A MECHANICAL MEANS OF DETERMINING THE VALUE OF "VOLUME" ON A BREAD SCORE REPORT

WILLIAM H. CATHCART

Research Department, American Institute of Baking, Chicago, Illinois

(Read at the Annual Meeting, May 1940)

Many ways are used for reporting the volume of bread. In scoring commercial bread some operators simply look at the loaf and give it a value, which is included in a numerical score. Others merely report cubical content (in cubic inches or cubic centimeters) exclusive of the numerical score, while still others have devised methods of including the number of cubic centimeters of loaf volume in a total numerical score. Saunders, Nichols, and Cowan (1922), Blish (1928, 1929), and Larmour (1929) have discussed the two latter methods.

The following method has proved to be a simple one for quite accurately employing a "yardstick" (determined mechanically) for volume on a bread score report.

Commercial Bread Scoring

In scoring commercial bread, volume is not given as much consideration as in experimental scoring. The numerical score which has been in use at the Institute since 1922 has been rather widely accepted. This scorecard was developed by considering other scorecards and by a questionnaire to all members of the American Bakers' Association for their ideas (see Baking Technology **1**: 14, 42 (1922). In this scorecard loaf volume is assigned a maximum of 10 points out of 100.

It is evident that a value for volume based on appearance would lead to great error. It is also evident that an attempt to arrive at a value for volume for various loaves on the basis of their total cubical volume would be erroneous, due to the great variations of total weight with total volume. However, the value for volume can be based quite accurately on the volume of a unit weight of the bread. This will be referred to hereafter as unit volume. This might be either cubic inches per ounce or cubic centimeters per gram. There are several reasons for preferring the former:

1. *The average unit volume (cu. in. per oz.) of many loaves scored comes fairly close to 10.* The average unit volume of the loaves scored in the last 18 months at the Institute is 9.5 cu. in. per oz. of bread. Pirrie (1936) reported unit volume on loaves from various parts of the country with an average of 10.2 cu. in. per oz. of bread.

2. *The correct amount of dough for a certain pan will yield a loaf whose volume is approximately 10 cu. in. per oz.* It is well known that for a certain type of bread too large or too small a pan will give a resulting loaf of relatively poorer quality than the more nearly correct size. What is the optimum size?

Ingles (1937) reported some collaborative work of the Research Committee on the A. S. B. E. on the effect of pan sizes on the palatability (eating qualities) of bread. Palatability was considered to consist chiefly of odor, taste, and mouth feel. The results of this report confirm earlier findings by Pickering and Norton (1933) that 5.83 cu. in. of pan size per ounce of dough (round-top bread) are the most desirable, or produce the most palatable loaf. It was also concluded that: "These experiments seem to show that for 30 hour old bread, the pan dimension, whether longer or shorter, does not matter so much, just so long as we keep the pan size at 5.83 cubic inches per ounce of dough—but that dropping this to even 5.50 noticeably affected palatability."

Cathcart (1937) tested the effect of various factors of "make-up" on bread flavor. Flavor here was considered as the aroma and the taste of the bread. Pan sizes varying from 4.43 cu. in. per oz. of dough to 7.19 cu. in. per oz. of dough were tested. The bread was one-pound round-top loaves. Although the differences found in the flavor of bread baked in the various sized pans was slight, the results of the experiments favored the pan having a capacity of 5.97 cu. in. per oz. of dough as being the most desirable.

An average of the above factors (optimum cu. in. of pan volume per oz. of dough) is about 5.9. Table I shows typical results with round-top

bread using this factor for various sizes of pans. It will be noted that unit volumes of all the hand-made samples are 10 or over, while the machine-made samples are 10 or very close to 10.

TABLE I

UNIT VOLUME OF ROUND-TOP BREAD RESULTING FROM VARIOUS SIZES OF PANS
Factor 5.9 used to calculate scaling weight of dough. Medium rich formula and good grade baker's patent flour used.

Exp. No.	Pan measurements in inches			Bread volume, cu. in. per oz.	Remarks
	Top inside	Bottom outside	Depth		
1	4½ × 9	3¾ × 8¼	3	10.5	Hand make-up
7	4½ × 9	3¾ × 8¼	3	10.5	Hand make-up
17	4½ × 9	3¾ × 8¼	3	10.7	Hand make-up
23	4½ × 9	3¾ × 8¼	3	9.9	Machine make-up
37	4½ × 9	3¾ × 8¼	3	10.1	Machine make-up
3	4⅞ × 8¾	4¼ × 8	2½	10.7	Hand make-up
9	4⅞ × 8¾	4¼ × 8	2½	10.1	Hand make-up
19	4⅞ × 8¾	4¼ × 8	2½	10.7	Hand make-up
25	4⅞ × 8¾	4¼ × 8	2½	10.2	Machine make-up
39	4⅞ × 8¾	4¼ × 8	2½	10.0	Machine make-up
4	4¾ × 11¼	4⅛ × 10¾	3	10.0	Hand make-up
10	4¾ × 11¼	4⅛ × 10¾	3	10.1	Hand make-up
20	4¾ × 11¼	4⅛ × 10¾	3	10.5	Hand make-up
26	4¾ × 11¼	4⅛ × 10¾	3	9.5	Machine make-up
40	4¾ × 11¼	4⅛ × 10¾	3	10.0	Machine make-up
11	4¼ × 8½	3½ × 8¼	2⅜	10.9	Hand make-up
14	4¼ × 8½	3½ × 8¼	2⅜	10.8	Hand make-up
21	4¼ × 8½	3½ × 8¼	2⅜	10.8	Hand make-up
30	4¼ × 8½	3½ × 8¼	2⅜	10.4	Machine make-up
34	4¼ × 8½	3½ × 8¼	2⅜	10.1	Machine make-up
41	4¼ × 8½	3½ × 8¼	2⅜	9.5	Machine make-up
6	4⅛ × 9¾	3½ × 9¼	2⅞	10.8	Hand make-up
12	4⅛ × 9¾	3½ × 9¼	2⅞	10.6	Hand make-up
22	4⅛ × 9¾	3½ × 9¼	2⅞	10.4	Hand make-up
28	4⅛ × 9¾	3½ × 9¼	2⅞	10.4	Machine make-up
42	4⅛ × 9¾	3½ × 9¼	2⅞	10.2	Machine make-up
13	4½ × 12	3¾ × 11½	2¾	10.7	Hand make-up
33	4½ × 12	3¾ × 11½	2¾	10.0	Machine make-up
16	4½ × 10	3¾ × 9½	2¾	10.5	Hand make-up
32	4½ × 10	3¾ × 9½	2¾	10.2	Hand make-up
36	4½ × 10	3¾ × 9½	2¾	10.1	Machine make-up

Realizing that proof time would greatly affect the unit volume, the loaves were proofed to a definite height (considered normal) above the top of the pan. The sponge and dough method of fermentation was used and the formula was as follows:

	%	
Sponge:		
Flour	60	
Water (approx.)	62	(Sp. abs.)
Yeast	1.75	(Total)
Malt extract	1	(60° Lintner)
Yeast food	0.25	
Dough:		
Flour	40	
Water (approx.)	63	(Total abs.)
Salt	2	
Corn sugar	6	
Milk solids	4	
Shortening	3.5	

The same flour was used for all of these tests.

Experiments also have been made using different flours and the same pan (top inside 10 in. × 4¼ in., bottom outside 9½ in. × 3¾ in., depth 2¾ in.). The factor 5.9 was used as before. The quality of the flours varied from fairly good to excellent, and hand make-up was employed. The volumes on the resulting bread varied from 9.9 to 10.9 cubic inches per ounce.

The most recent work on commercial pan sizes and shapes was done by Cathcart *et al.* (1938, 1939).[1] This research determined the best pan shape and size for a 20-ounce loaf of twist bread and round-top bread. All of the experiments were handled on a commercial scale, were machine molded, and were completely scored. Thirty experiments on round-top bread made in the optimum pan (using optimum scaling weights and normal proof) yielded volumes from 9.0 to 9.9 cu. in. per oz. of bread. A similar series on twist bread showed a variation in volume from 9.3 to 10.3 cu. in. per oz. of bread.

3. *Loaves of bread which score highest have a volume of about 10 cu. in. per oz.* Pirrie (1936) has stated that " in the opinion of at least one service agency with national contact in this respect, bread with a unit volume of 10 or a loaf volume of 160 cubic inches for bread weighing one pound, averages the highest score."

Cathcart, Walmsley, and Habenicht (1938) found that, on the average, loaves with a volume nearest to 10 cu. in. per oz. of bread scored the highest.

In order to test this further, experiments were made with a pan (top inside 10⅝ × 5, bottom outside 10⅛ × 4⅜, depth 2¾ in.) that produced optimum results with both twist and round-top bread (Cathcart *et al.* 1938, 1939). The optimum amount of dough (handled same as Cathcart and co-workers) was scaled into pans of this size and the dough proofed for various lengths of time to vary the unit volume. The

[1] The optimum number of cubic inches of pan volume per ounce of dough is given by Cathcart and co-workers. The figure is different for each pan depth: 5.99, 5.46, and 5.30 cu. in. per oz. of dough for the 3 in., 2¾ in. and 2½ in. depth respectively. These data were not available when the factor 5.9 was used for experiments reported in Table I.

loaves were then completely scored. Typical results for round-top bread are given in Table II and for twist bread in Table III.

TABLE II

ROUND-TOP BREAD MADE IN OPTIMUM PAN WITH OPTIMUM SCALING WEIGHT—PROOF TIME VARIED

Experiment No.	312	313	314	315	316	317	318	319
Volume (cu. in. per oz.)	7.5	8.8	9.5	10.0	10.0	9.5	10.9	11.1
Minutes proof	40	45	50	55	60	65	70	75
Total score	83.5	87.3	89.0	91.5	92.5	90.5	87.1	83.9
Eating quality	Fair	Fair	Good	Best	Good	Fair	Poor	Poor

TABLE III

TWIST BREAD MADE IN OPTIMUM PAN WITH OPTIMUM SCALING WEIGHT—PROOF TIME VARIED

Experiment No.	304	305	306	307	308	309	310	311
Volume (cu. in. per oz.)	9.0	9.3	9.5	10.0	10.2	9.9	10.6	10.8
Minutes proof	40	45	50	55	60	65	70	75
Total score	91.5	91.7	92.0	92.5	92.3	92.4	91.9	90.7
Eating quality	Good	Good	Good	Best	Good	Fair	Fair	Fair

It will be noted from Tables II and III that the loaves having optimum "Total Score" and "Eating Quality" have a volume of 10. Considering all of the experiments, volumes from about 9.3 to 10.3 produced good-quality bread.

Discussion

From the above it is seen that the optimum volume is very close to 10 cubic inches per ounce of bread and that commercial bread can have too large a volume as well as too small a volume.

It is realized that the optimum loaf volume varies from territory to territory, in accordance with prevailing desires. However, it has been found that too large or too small loaves generally suffer on other points in the bread score, especially on internal characteristics (grain, color of crumb, and texture). Again, when one has to judge bread from all parts of the country, it is a practical impossibility for one to have standards for every section of the country; not only do desired volumes vary within a state, but many times within cities. Lastly, what use is a bread score, anyway? It is not to show how nearly perfect a loaf is, but to serve as a guide to how the baker's bread is today as compared to yesterday, last week, etc. Thus, a standard of 10 will serve very nicely for the whole country, and from experience in these laboratories, it will do it with less error than attempting to set up standards (either in one's mind or on paper) for various sections of the country.

Any value for volume up to 10, measured in cubic inches per ounce of bread, can be recorded directly on the score. Any volume above 10 can be recorded full value (10) on the score, or *better* be degraded by the same amount that it is over 10.

However, a scale which may be followed is given in Table IV.

TABLE IV

VOLUME AND SCORE

Cubic inches per ounce bread by measurement	Value given on score
11.5 to 12.4	8
10.5 to 11.4	9
9.5 to 10.4	10
8.5 to 9.4	9
7.5 to 8.4	8
6.5 to 7.4	7
5.5 to 6.4	6

Experimental Bread Scoring

Although very few data are available, it seems that experimental bread scoring might be handled in the same way as commercial bread scoring with one modification. Saunders *et al.* (1922), Larmour (1929), and others have indicated that the value for loaf volume should represent 40% to 60% of the experimental score; thus it is necessary to multiply the cubic inches per ounce of bread by 4 if the volume is to represent 40% of the total score, etc. The average volume for 25 tests on five different high-grade patent flours was 9.2 cu. in. per oz. of bread made in both the tall and low form pans according to A. A. C. C. procedure.

Experimental loaves baked according to the procedure used at the Institute,[2] yielded an average volume of 9.6 cu. in. per oz. of bread when high-grade patent flour was used.

Summary

Evidence is presented which indicates that unit volume (cubic inches per ounce of bread) can be used directly on a commercial bread score, and that a definite multiple of it can be used on an experimental score.

Acknowledgment

The author wishes to acknowledge the assistance of members of the baking and allied industries in reviewing the manuscript.

[2] Pan size was: top inside 3½ in. × 6 in., bottom outside 3 in. × 5½ in., and depth 3 in. The amount of dough (very lean formula) scaled in these pans was approximately 325 g. (some variation due to varying scaled weight with flour absorption).

Literature Cited

Blish, M. J.
1928 Proposed reporting system for standard baking test. Cereal Chem. **5**: 289–294.
1929 Reporting system for the standard experimental baking test. Cereal Chem. **6**: 253–258.
Cathcart, W. H.
1937 Experiments in determining bread flavor. Cereal Chem. **14**: 735–751.
—— Walmsley, W., and Habenicht, A.
1938 Pan size factor methods. The A.B.A. Monthly Bulletin **2**: 140–143.
1938 The A.B.A. Monthly Bulletin **2**: 243 and 273.
—— Pushnik, W., and Walmsley, W.
1939 The A.B.A. Monthly Bulletin **2**: 331, 358, 394, 424, and 456.
Ingles, B.
1937 Effect of pan size on palatability of bread. Proceedings, A.S.B.E., pp. 41–44.
Larmour, R. K.
1929 A single figure estimate of baking scores. Cereal Chem. **6**: 164–174.
Pickering, C. S., and Norton, S.
1933 Effect of pan shapes on loaf characteristics. A.S.B.E. Bulletin No. 90.
Pirrie, P. G.
1936 Size of loaf—what does it mean to the baker and the consumer? Baker's Weekly, Oct. 17, p. 41.
Saunders, C. E., Nichols, R. W., and Cowan, P. R.
1922 Researches in regard to wheat, flour and bread. Dominion of Canada, Dept. of Agr. Bull. 97.

BETA-AMYLASE ACTIVITY AND ITS DETERMINATION IN GERMINATED AND UNGERMINATED CEREALS[1]

ERIC KNEEN and R. M. SANDSTEDT

Department of Agricultural Chemistry, University of Nebraska, Lincoln, Nebraska

(Received for publication August 6, 1940)

It is generally recognized that the hydrolytic action of malt on soluble starch results in the production of sugars. The amylase component of malt which can readily bring about this sugar production has been designated the saccharogenic amylase or beta-amylase. Logically therefore this type of amylase activity has been measured by determining the amount of reducing substances produced in a given time or conversely the amount of starch converted into reducing substances. It is recognized that these reducing substances are frequently a mixture of dextrins, glucose, and maltose. However, because of the predominance of maltose in the reaction products the hydrolysis is usually expressed arbitrarily in terms of maltose production.

Most investigators have recognized that the dextrinogenic or alpha component of malt amylase likewise has a degree of saccharogenic activity. Lack of an adequate method for distinguishing between the

[1] Published with the approval of the Director as paper No. 271, Journal Series, Nebraska Agricultural Experiment Station.

saccharifying activities of the two components has resulted in conclusions such as the rather indefinite one that "the contribution made by the alpha-amylase is probably relatively small" (Sallans and Anderson, 1938, p. 408). It was with the purpose of devising a method for the accurate determination of the relative roles of alpha- and beta-amylase in saccharification that the investigation herewith reported was undertaken.

Methods for Determining Beta-Amylase Activity

Two methods of approach have been used in attempts to differentiate between the saccharifying activities of the two malt amylase components. The one most generally adopted has been the differential destruction of the alpha component by such technique as that of Ohlsson (1926, 1930). This has not proved entirely satisfactory and as yet is far from quantitative.

Blom, Bak, and Braae (1937) suggested that the saccharifying actions of alpha- and beta-amylase may be differentiated on the basis of a time-temperature inactivation curve for the malt extract. By progressive heating past the point at which complete destruction of the beta-amylase was effected they found a straight-line relationship between time of heating and saccharifying activity of the remaining alpha component. By extrapolation to zero time of heating the alpha-saccharogenic activity of the unheated extract was determined. Subtraction of this value from the saccharifying activity of the original extract resulted in a value which they postulated as due to beta-saccharifying activity. To those familiar with partial heat inactivation of malt extracts this suggested method has obvious flaws from the standpoint of its application in routine quantitative procedure. It is well known that the sensitivity of alpha-amylase to high temperatures is dependent upon a number of subsidiary factors, among which are extent of dilution, reaction of the extract, and the presence and concentration of certain ions. Too, the above method does not have the speed and simplicity desirable in a routine technique.

An ideal method for differential determination of the saccharifying activities of the two components would require no destructive technique and would have the simplicity and reliability necessary for routine procedure. The method previously devised by Sandstedt, Kneen, and Blish (1939) for the determination of alpha-dextrinogenic activity in the presence of beta-amylase and the reports of several authors that alpha- and beta-amylase are roughly additive in saccharification suggested that this might be possible and led to the development of the proposed method.

Method of Measuring Saccharification

In order to investigate the differential activities of the malt amylases a standard set of conditions for saccharification was necessary. The conditions finally adopted and followed throughout the investigation herein reported were as follows: to 20 cc. of 2% buffered soluble starch solution [2] in a 100-cc. Erlenmeyer flask is added sufficient water so that the final enzyme addition brings the total volume to 30 cc. When the flask contents have come to temperature in the 30° C. bath the enzyme is added and the hydrolysis allowed to proceed for exactly 15 minutes. At the end of the 15 minutes the reaction is stopped by the addition of 20 cc. of 1% sulfuric acid from a fast-flowing pipette As the acid is added the reaction mixture is agitated for rapid mixing. Shortly following the acid addition a 5-cc. aliquot of the solution is transferred to a test tube containing 10 cc. of 0.10*N* ferricyanide solution and reducing value determined by the method of Blish and Sandstedt (1933) as modified for 0.10*N* reagents by Sandstedt (1937).

The above technique has several advantages. The short period of hydrolysis permits great rapidity of determination; by starting the runs at two-minute intervals eight hydrolyses may be completed with ease in 30 minutes of total elapsed time. The addition of the sulfuric acid not only terminates the hydrolysis abruptly, but likewise its presence appears to have an as yet inexplicable stabilizing influence on the relationship between maltose present and volume of ferricyanide reduced.

Conversion of Ferricyanide Reduced to Maltose Equivalents

It is recognized that the reducing substance resulting from the action of malt extracts on starch may not be exclusively maltose. However the reducing products have been almost universally reported as maltose equivalents. For convenience and lack of a more suitable designation that practice was followed in the experiments herein reported. It became necessary therefore to construct a table to convert cc. of ferricyanide reduced under the prescribed conditions to mg. maltose. Table I is the conversion table set up for this purpose and may be used as a standard when the experimental conditions are as stipulated. This table was prepared by using an especially prepared sample of maltose of exceptional purity. Conditions characteristic of the experimental technique, such as the buffer and sulfuric acid concentration, were necessarily adjusted to an equivalent basis. It should be emphasized that the conversion data of Table I may be used

[2] Buffered soluble starch solution: Prepare a suspension of 10 g. (dry weight) of Merck's Lintner soluble starch and pour slowly into boiling water. Cool, add 25 cc. of buffer solution, bring to 500 cc. volume with water and saturate with toluol. The stock buffer solution is 120 cc. glacial acetic acid and 164 g. anhydrous sodium acetate made up to 1000 cc. with water.

TABLE I

FERRICYANIDE-MALTOSE CONVERSION TABLE FOR THE DETERMINATION OF MALT-SACCHAROGENIC ACTIVITY

0.10*N* ferricyanide reduced	Maltose	0.10*N* ferricyanide reduced	Maltose
cc.	*mg.*	*cc.*	*mg.*
0.50	1.46	5.50	16.57
1.00	2.92	6.00	18.20
1.50	4.38	6.50	19.85
2.00	5.85	7.00	21.50
2.50	7.32	7.50	23.15
3.00	8.79	8.00	24.88
3.50	10.28	8.50	26.70
4.00	11.83	9.00	28.65
4.50	13.40	9.50	30.65
5.00	14.97	—	—

only when the above experimental conditions are satisfied. Other conditions such as those involving a change in kind or concentration of buffer or the absence of sulfuric acid would necessitate a different conversion table.

To conserve space the data of Table I are presented in abbreviated form. It is suggested that for routine laboratory use the data be plotted on large graph paper from which the maltose equivalents may be read with ease. It should be noted that the maltose corresponding to a found volume of ferricyanide reduced is the amount present in the 5-cc. aliquot taken for reduction. For example if the titration indicates 5.00 cc. of ferricyanide reduced the 5-cc. aliquot contains 14.97 mg. of maltose. The total amount of maltose produced, the amount in 50 cc. total volume, was then 10×14.97 or 149.7 mg. If it is desired to express this as starch the conventional factor of 0.95 is used to give 142.3 mg. starch converted to maltose in 15 minutes.

Relationship between Alpha-Dextrinogenic and Alpha-Saccharogenic Activity

Sandstedt, Kneen, and Blish (1939) demonstrated that alpha-dextrinogenic activity could be accurately measured in the presence of beta-amylase. It has proved possible to transpose such quantitative dextrinogenic values into equivalent saccharogenic activity; *i.e.*, from a given dextrinization value the apparent saccharification that this amount of alpha-amylase would produce may be calculated.

The data relating alpha-dextrinogenic activity to alpha-saccharogenic activity are presented in Table II. The dextrinogenic activity is given in reciprocal minutes, the saccharifying activity in mg. maltose. A value listed under reciprocal minutes is that found for a given

TABLE II

RELATIONSHIP OF SACCHAROGENIC ACTIVITY TO THE DEXTRINOGENIC ACTIVITY OF ALPHA-AMYLASE

Alpha-dextrinogenic activity	Alpha-saccharogenic activity	Alpha-dextrinogenic activity	Alpha-saccharogenic activity
1/min.	*mg. maltose*	*1/min.*	*mg. maltose*
0.010	3.17	0.160	46.93
0.020	6.34	0.170	49.39
0.030	9.49	0.180	51.80
0.040	12.66	0.190	54.20
0.050	15.83	0.200	56.58
0.060	18.93	0.210	58.89
0.070	22.01	0.220	61.17
0.080	25.02	0.230	63.37
0.090	27.93	0.240	65.48
0.100	30.74	0.250	67.50
0.110	33.52	0.260	69.42
0.120	36.31	0.270	71.33
0.130	39.05	0.280	73.25
0.140	41.74	0.290	75.17
0.150	44.38	0.300	77.02

amount of alpha-amylase when dextrinizing activity is determined by the technique of Sandstedt, Kneen, and Blish (1939) using an excess of beta-amylase. The corresponding value given for mg. maltose is the apparent maltose produced in 15 minutes by an equal amount of alpha-amylase when it saccharifies starch under the prescribed conditions. For example if 0.05 gram equivalent of alpha-amylase indicates a dextrinizing activity of 0.10 reciprocal minutes (10 minutes), 0.05 gram equivalent would give an apparent saccharification of 30.7 mg. maltose.

The data presented in Table II were established by repeated runs with several samples of alpha-amylase prepared from barley and wheat malts by the Ohlsson (1930) technique. As far as could be determined the preparations were free from beta-amylase activity. For instance prolonged heating of the extracts at 70° C. in an attempt to inactivate any possible remaining traces of beta-amylase resulted in no significant change in the relationships recorded in Table II.

The Saccharifying Activity of Alpha- and Beta-Amylases, Individually and in Combination

In order to compare the saccharifying activity of two enzymes or of more than one concentration of the same enzyme it is important that certain limitations be imposed upon the degree of saccharification permitted. For instance the fact that extracts of ungerminated grains tend to reach a common level of starch hydrolysis at about 60% conversion necessitates a saccharification limit of not more than this

figure. An attempt was made to more clearly define these limits. The data are presented in Figures 1 and 2.

Figure 1 illustrates the progress of starch hydrolysis under the influence of increasing concentrations of beta- and alpha-amylases acting independently. Two beta-amylase extracts were used, one from ungerminated barley, the other from ungerminated wheat. Similarly two samples of alpha-amylase, one prepared from barley malt and the other from wheat malt, were used. The differences in activity between the barley and wheat extracts have no general significance, being

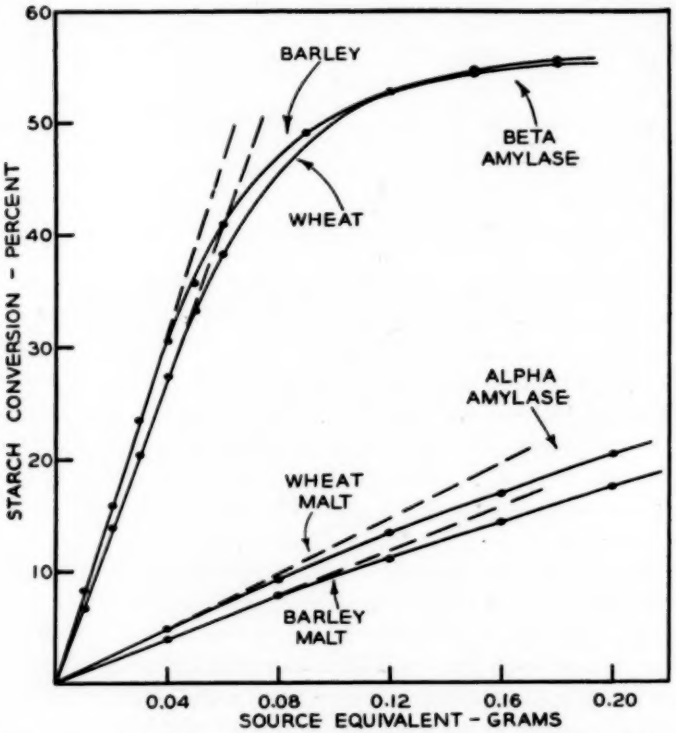

Fig. 1. Relationship of individual amylase concentration to starch saccharification.

characteristic of the individual samples only. The solid lines conform to the found data. The broken lines represent the theoretical progress, assuming perfect linearity.

The curves of Figure 1 illustrate that, under the conditions of the experiment, the relationship between beta-amylase concentration and conversion of the starch to maltose is linear up to approximately 30% conversion. It is apparent then that when determining the saccharifying activity of ungerminated wheat or barley the enzyme concentration

should be so regulated that this limit of 30% is not exceeded in the 15-minute run. The curves of Figure 1 relating alpha-amylase concentration to apparent saccharification indicate that there is a lack of linearity over the whole range. Since saccharification is rarely used as a measure of alpha-amylase activity this does not present a serious problem.

The curves of Figure 2 illustrate the relationship between con-

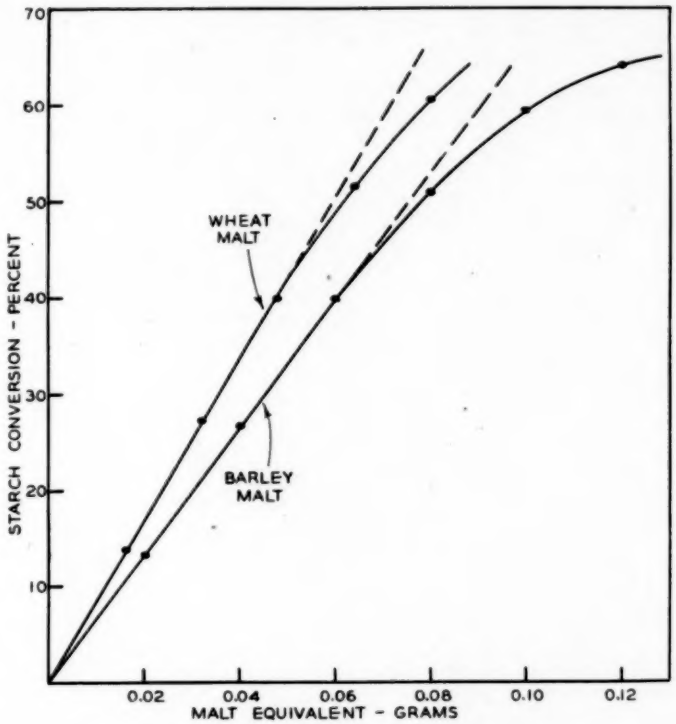

Fig. 2. Relationship of malt concentration to starch saccharification.

centration of unmodified malt and conversion of starch to maltose. Here too the broken lines are extensions of the previous linear relationship and as with the data of Figure 1 the difference in activity between wheat and barley malt is not characteristic of all such malts.

The data recorded in Figure 2 demonstrate that a linear relationship holds only up to the point where approximately 40% of the starch has been converted to maltose.[3] When determining the saccharifying activity of malt extracts it is then important that conversion be not carried past this limiting value.

[3] The data therefore conform to Kjeldahl's law of proportionality. Apparently this limitation has been neglected in some of the more recent researches on malt amylase.

The Additive Nature of Alpha- and Beta-Amylase in Saccharification

It was demonstrated above that when alpha-amylase is caused to saccharify starch in the absence of beta-amylase the apparent saccharification may be calculated from predetermined alpha-dextrinization values. Now if alpha- and beta-amylase should prove to be additive in saccharification, that is if the saccharification produced by a mixture of the two amylases should coincide with the summation of the independent actions, then by subtraction the saccharification due to beta-amylase alone could be calculated.

Ohlsson (1930) and Ohlsson and Uddenberg (1933) found that alpha- and beta-amylases were roughly additive in their action on soluble starch. In further work Stenstam, Björling, and Ohlsson (1934) found that this additive effect was true only for certain ratios of alpha- to beta-amylase. In a more adequate treatment Freeman and Hopkins (1936) reported that the starch-saccharifying activities of the two malt amylase components were additive at low enzyme concentrations but not at high concentrations nor when the beta-amylase concentration was high relative to alpha. Hills and Bailey (1938) reported that, when using purified enzymes, "the amount of maltose produced by alpha- and beta-amylases acting together was approximately the sum of the two amounts produced by their acting separately."

For investigation of the possible quantitative nature of the additive relationship of alpha- and beta-amylase in saccharification a number of determinations were made covering a wide range both of total enzyme concentration and of the ratios of beta- to alpha-amylase. Maltose produced by the independent actions and likewise by the combined action of the two components added simultaneously was measured. The alpha-amylase preparations were obtained from both barley and wheat malts by the Ohlsson (1930) technique and showed no measurable beta-amylase activity. The beta-amylase preparations were hard winter wheat extracts of determined freedom from alpha-amylase.

The data dealing with the additive nature are presented in Table III. The values are arranged in descending order of magnitude of the ratio of beta- to alpha-amylase. These ratios range from one in which the saccharifying activity of beta-amylase was more than 50 times that of alpha to a ratio in which the alpha-amylase activity was more than 20 times that of beta. The range of total concentrations covered varied from a high of 166 mg. maltose produced (39.4% starch conversion) to a low of 21 mg. maltose (4.99% conversion). All activity data are expressed as mg. maltose produced in 15 minutes under the specified conditions.

Table III lists "found" data for the saccharifying activities of the

TABLE III

RELATIONSHIP OF THE RATIO OF ALPHA- AND BETA-AMYLASE IN COMBINATION TO THE ADDITIVE NATURE OF THEIR ACTIVITIES

Alpha-amylase activity	Beta-amylase activity	Ratio: beta to alpha	Alpha plus beta: found	Alpha plus beta: calculated	Ratio: found to calculated	Deviation: found from calculated
mg. maltose	*mg. maltose*		*mg. maltose*	*mg. maltose*		%
3.0	151.0	50.30	155.5	154.0	1.010	0.97
4.0	155.0	38.75	166.0	159.0	1.044	4.40
3.0	81.0	27.00	83.0	84.0	0.988	1.19
4.0	89.0	22.25	94.0	93.0	1.011	1.08
3.0	44.5	14.83	46.0	47.5	0.968	3.16
9.0	128.0	14.23	139.0	137.0	1.015	1.46
4.0	53.5	13.38	58.5	57.5	1.017	1.74
9.0	83.5	9.28	93.0	92.5	1.005	0.54
13.0	89.0	6.85	103.5	102.0	1.015	1.47
4.0	27.0	6.75	30.0	31.0	0.968	3.23
9.0	60.6	6.73	69.0	69.6	0.991	0.86
3.0	16.5	5.50	21.0	19.5	1.077	7.69
9.0	49.2	5.47	57.0	58.2	0.979	2.06
19.0	97.0	5.11	119.0	116.0	1.026	2.59
27.0	128.0	4.74	153.0	155.0	0.987	1.29
9.0	37.8	4.20	45.5	46.8	0.972	2.78
13.0	53.5	4.12	68.0	66.5	1.023	2.26
25.0	89.0	3.56	114.5	114.0	1.004	0.44
18.0	60.6	3.37	79.0	78.6	1.005	0.51
18.5	62.0	3.35	81.0	80.5	1.006	0.62
27.0	83.5	3.09	109.0	110.5	0.986	1.36
9.0	26.5	2.95	37.0	35.5	1.042	4.23
9.0	25.1	2.79	35.5	34.1	1.042	4.11
18.0	49.2	2.73	67.0	67.2	0.997	0.30
19.0	43.0	2.26	62.0	62.0	1.000	0.00
25.0	53.5	2.14	79.5	78.5	1.013	1.27
18.0	37.8	2.10	54.0	55.8	0.968	3.23
13.0	27.0	2.08	43.0	40.0	1.075	7.50
Av.					1.008	2.23
49.0	97.0	1.980	137.0	146.0	0.938	6.17
45.0	83.5	1.855	121.0	128.5	0.941	5.84
51.5	85.0	1.651	132.0	136.5	0.967	3.30
9.0	12.9	1.433	23.0	21.9	1.050	5.02
18.0	25.1	1.394	45.0	43.1	1.044	4.41
25.0	27.0	1.080	51.0	52.0	0.981	1.92
27.0	26.5	0.981	57.0	53.5	1.065	6.54
49.0	43.0	0.877	87.0	92.0	0.946	5.44
18.0	12.9	0.717	31.0	30.9	1.003	0.32
45.0	26.5	0.589	71.0	71.5	0.993	0.70
51.5	25.5	0.495	82.0	77.0	1.065	6.49
51.5	14.5	0.282	71.0	66.0	1.076	7.57
94.5	25.5	0.270	112.5	119.0	0.945	5.46
94.5	14.5	0.154	108.0	109.0	0.991	0.92
51.5	4.5	0.087	58.0	56.0	1.036	3.57
94.5	4.5	0.048	99.0	99.0	1.000	0.00
Av.					1.003	3.98
Av. for all determinations					1.006	2.86

enzymes individually and in combination. The summations of the two individual activities are listed as "calculated" activities. Two methods of comparing the "found" and "calculated" results are used. The data for the ratio of found to calculated express the degree to which the two values satisfy a one-to-one ratio; those for the deviation between found and calculated values indicate the magnitude of error which might be anticipated in the application of such technique. It should be emphasized that because of the nature of the operations necessary to obtain the data of Table III the probability of error is greater than would be anticipated in actual practice. The 44 runs are divided into two groups: 28 runs in which the ratios of beta-amylase activity to alpha-activity are above 2.00, and the remaining 16 in which this ratio is below 2.00. Average ratios and percent deviations between "found" and "calculated" data are given for each group as well as for all 44 runs.

The data indicate that there is no consistent deviation from a one-to-one ratio of the found and calculated results; the average ratio for all 44 runs was 1.006. The percentage of deviation between the two values is not serious throughout but does appear to be somewhat less for the group in which beta-amylase is high relative to alpha. The average deviation for the 28 runs in which the ratio of beta- to alpha-amylase was above 2.00 is 2.23% as compared to 3.98% for those ratios below 2.00.

Using the amount of maltose produced in the "found" column as a measure of the enzyme concentration, the data of Table III may be used to illustrate the relationship of total concentration to the additive nature of the two components. In Table IV the data for the 28 runs where the ratio of beta- to alpha-amylase was greater than 2.00 are arranged into three groups: (1) eight combinations from which the maltose produced was above 100 mg., (2) ten combinations from which the maltose produced was between 100 and 60 mg., and (3) ten from which the maltose produced was less than 60 mg.

It is apparent from Table IV that the closest agreement between the found data and the calculated is in the intermediate group. Here the average percentage of deviation is less than one, as compared to 1.75% deviation for the high and 3.97% for the low group. However it is evident that relatively high concentrations of the enzymes do not destroy the relationship as indicated by the reasonably low error of 1.75% in the high group. The average deviation of approximately 4% for the group of low concentrations appears more serious. However this would seem to be nothing more than an indication that measurements of low degrees of saccharification are subject to a relatively greater percentage of error than measurements made at higher levels.

TABLE IV

RELATIONSHIP OF TOTAL ENZYME CONCENTRATION TO THE ADDITIVE NATURE OF ALPHA- AND BETA-AMYLASE ACTIVITY

Group (1): maltose above 100 mg.		Group (2): maltose between 60 and 100 mg.		Group (3): maltose below 60 mg.	
Maltose produced	Deviation: found from calculated	Maltose produced	Deviation: found from calculated	Maltose produced	Deviation: found from calculated
mg.	%	*mg.*	%	*mg.*	%
166.0	4.40	94.0	1.08	58.5	1.74
155.5	0.97	93.0	0.54	57.0	2.06
153.0	1.29	83.0	1.19	54.0	3.23
139.0	1.46	81.0	0.62	46.0	3.16
119.0	2.59	79.5	1.27	45.5	2.78
114.5	0.44	79.0	0.51	43.0	7.50
109.0	1.36	69.0	0.86	37.0	4.23
103.5	1.47	68.0	2.26	35.5	4.11
—	—	67.0	0.30	30.0	3.23
—	—	62.0	0.00	21.0	7.69
Av.	1.75	Av.	0.86	Av.	3.97

The data of Table III permit the conclusion that alpha- and beta-amylase are additive in saccharification throughout a wide range of ratios of beta to alpha, and particularly so when the concentration of beta-amylase is high relative to alpha. In addition, as arranged in Table IV, the data illustrate that, at least below the saccharification level represented by 40% starch conversion, total concentration of the enzymes does not materially affect the additive relationship.

The Determination of Beta-Amylase Activity

Data presented above indicate that alpha- and beta-amylase are additive in saccharification and that the saccharifying activity of alpha-amylase may be readily calculated from the determination of its absolute dextrinizing activity. This dextrinizing activity may be determined by the routine procedure of Sandstedt, Kneen, and Blish (1939). It becomes then a simple matter to determine the absolute beta-saccharogenic activity of a malt extract.

The steps in the determination of beta-amylase activity follow in order:

1. Determine the minutes required for dextrinization when using the alpha-amylase method of Sandstedt, Kneen and Blish (1939).

2. Using the method for determining saccharification outlined above find the total mg. of maltose produced in 15 minutes. For this determination the precaution must be taken that the concentration of malt used does not give greater starch hydrolysis than the limiting 40% conversion.[4]

[4] Malt concentrations which may be used with a resultant starch conversion safely below the 40% level vary from the equivalent of 0.01 g. for malts of very high diastatic power to 0.05 g. for low diastatic malts.

3. Calculate in reciprocal minutes the dextrinization time for the amount of alpha-amylase present in the malt sample used for saccharification. This value may be found by multiplying the reciprocal of the time in minutes found in the alpha-amylase determination by the factor:

$$\frac{\text{Malt equivalent used for saccharification (g.)}}{\text{Malt equivalent used for alpha-dextrinization (g.)}}$$

If the alpha-amylase activity is already recorded as the alpha-amylase units of Sandstedt, Kneen, and Blish (1939) the desired reciprocal minutes for the amount of alpha-amylase acting in saccharification may be derived by multiplying the figure for alpha-amylase units by the factor:

$$\frac{\text{Malt equivalent used for saccharification (g.)}}{0.4 \times 60}$$

4. Using Table II find the mg. maltose corresponding to the reciprocal minute value for the alpha-amylase taking part in saccharification. Subtract this value from the total mg. of maltose produced in the 15-minute saccharification. The difference represents the mg. of maltose produced by the beta-amylase present in the malt sample.

5. Beta-amylase units may now be calculated as the number of grams of starch converted to maltose by the beta-amylase of one gram of malt in one hour at 30° C.

The application of the above technique to data found for a barley-malt sample perhaps best illustrates the procedure. The pertinent data for one malt follow:

Dry matter content	93.07%
Alpha-dextrinization time for 0.05 g. equivalent	14.0 min.
Saccharification by 0.03 g. equivalent: ferricyanide reduced by 5 cc. aliquot less starch blank	4.26 cc.

Using these data the following calculations are made:

Maltose produced by 0.03 g. equivalent (Table I)	126.5 mg.
Alpha-dextrinization time for 0.03 g. equivalent	$\frac{0.03}{0.05} \times \frac{1}{14} = 0.0429$ reciprocal min.
Maltose due to alpha-amylase activity (Table II)	13.6 mg.
Maltose due to beta-amylase activity	$126.5 - 13.6 = 112.9$ mg.
Starch converted to maltose by beta-amylase	$112.9 \times 0.95 = 107.3$ mg.
Starch conversion in one hour	$0.1073 \times \frac{60}{15} = 0.429$ g.
Dry weight of malt equivalent used for saccharification	$\frac{93.07}{100} \times 0.03 = 0.0279$ g.
Starch converted to maltose by the beta-amylase of one gram of malt in one hour at 30° C. (beta-amylase units)	$\frac{0.429}{0.0279} = 15.38$ g.

That is, the malt has a beta-amylase activity of 15.4 units.

Definition of " Units "

In the past the arbitrary calculations commonly used to express saccharogenic activity, such as "degrees Lintner," have been meaningless from the standpoint of any true definition of the nature and extent of the hydrolysis taking place. Sandstedt, Kneen, and Blish (1939) suggested that the logical terminology for defining alpha-amylase activity is in terms of grams of starch dextrinized by one gram of malt in one hour. There is no logical reason why saccharogenic activity should not be expressed in a similar manner. Accordingly the following uniform terminology has been adopted and used throughout this communication where absolute and comparative definition of amylase activity is desired. The definition for alpha-dextrinogenic activity is included for comparison.

Malt-saccharogenic units.—The number of grams of soluble starch converted to maltose by one gram of malt in one hour at 30° C.

Beta-saccharogenic units (*beta-amylase units*).—The number of grams of soluble starch converted to maltose by the beta-amylase of one gram of malt in one hour at 30° C.

Alpha-dextrinogenic units (*alpha-amylase units*).—The number of grams of soluble starch which, under the influence of an excess of beta-amylase, are dextrinized by one gram of malt in one hour at 30° C.

The above unit values are calculated on the dry-weight basis throughout. The specifications as outlined for each determination must be followed exactly. Too, it should be emphasized again that a limit of 40% starch conversion should not be exceeded in the malt saccharification and 30% in the case of saccharification by the beta-amylase of ungerminated cereals such as barley and wheat.

Application of the Beta-Amylase Method

A group of five barley malt grains was selected to demonstrate the application of the beta-amylase method. These grains were ground finely in a burr mill and one gram of meal extracted with 100 cc. of water for one hour at 30° C. The extracts were centrifuged for five minutes and then poured rapidly through cotton plugs. Five-cc. aliquots of the extracts (equivalent to 0.05 g. malt) were used for the determination of alpha-amylase, the range of dextrinization times being from 14.0 to 22.25 minutes. For saccharification 3-cc. aliquots (equivalent to 0.03 g. malt) were used and gave a range of hydrolysis from 9.63% to 30.05% starch conversion. The results obtained are given in Table V. Data are presented for alpha-dextrinogenic units, malt-saccharogenic units, and for beta-saccharogenic units. In addition the fraction of the saccharification due to beta-amylase alone is illustrated.

TABLE V

STARCH-DEGRADING PROPERTIES OF VARIOUS MALTS

Malt	Alpha dextrinization	Malt saccharification	Beta saccharification	Fraction of saccharification due to beta-amylase
	Units	*Units*	*Units*	%
1	36.8	17.21	15.38	89.4
2	35.6	14.25	12.45	87.4
3	25.1	12.20	10.95	89.8
4	41.1	9.02	6.97	77.3
5	23.1	5.50	4.34	78.9

The data of Table V permit several significant conclusions. There is a high degree of correlation between the saccharifying activity of a malt and its beta-amylase content. On the other hand there seems to be no necessary correlation between the alpha-amylase activity and either the saccharifying activity or the beta-amylase activity of a malt. For example malt No. 4 has the highest alpha-amylase activity but is next to the lowest in both saccharification and beta-amylase activity. The alpha-amylase content of a malt obviously influences its saccharifying activity, the degree of this influence varying with different malts. With three of the five malts illustrated more than 10%, and with the remaining two more than 20%, of the saccharification may be attributed to the alpha-amylase content. It would seem that such differential methods as outlined above are essential in any attempt to gain an accurate conception of the roles played by the components of malt amylase in starch saccharification.

Summary

The saccharifying power of a malt extract either unmodified or modified in such a manner as to inactivate most of the alpha-amylase is commonly used as a measure of beta-amylase activity. It is generally recognized that alpha-amylase contributes to malt saccharification, the degree of that contribution being of uncertain magnitude. In order to obtain an accurate determination of the beta-amylase present it is necessary therefore to take cognizance of the presence of alpha-amylase and the extent of its influence on the saccharifying activity.

A rapid technique for measuring saccharifying activity was developed. This procedure involves a 15-minute digestion followed by acidification to halt the enzymic activity. Maltose production is measured by the ferricyanide method. A conversion table relating cc. of ferricyanide reduced to mg. maltose present is provided.

It was found that the saccharogenic activity of alpha-amylase was proportional to its dextrinogenic activity. From absolute alpha-

dextrinogenic values measured by the method of Sandstedt, Kneen, and Blish (1939) and simultaneously run alpha-saccharogenic activities, the relationship between these values was determined. A conversion table relating saccharogenic power to previously determined alpha-dextrinogenic activity is presented.

The limiting percentage of starch conversion below which there exists a linear relationship between enzyme concentration and starch saccharification was determined for the individual amylase components and for unmodified malt extract. For malt extract the relationship is linear up to 40% starch conversion and for beta-amylase alone up to 30% conversion. For alpha-amylase alone there appears to be no definite region of linearity.

The additive nature of alpha- and beta-amylase in saccharification was investigated over a wide range of combinations covering various total concentrations and various ratios of beta- to alpha-amylase. It was demonstrated that, within the limits of the experiment, an additive relationship holds throughout. Combinations in which the beta-amylase was low relative to alpha showed slightly more deviation from this relationship than did other combinations. In combinations where the saccharogenic power of the beta component is more than twice that of the alpha component, a condition generally characteristic of malts, the saccharogenic activity of a mixture of alpha- and beta-amylases is the sum of the individual activities of the two acting independently. The activity of the beta-component of malt may then be determined by subtracting from the total saccharogenic value the previously calculated alpha-saccharogenic value. These calculations may be performed with only slightly less accuracy when the ratio of beta- to alpha-saccharogenesis is less than 2.00.

Unit definitions are suggested for malt-saccharogenic activity and for beta-saccharogenic activity. These units, as were those proposed for alpha-dextrinogenic activity, have as their basis the number of grams of starch hydrolyzed by one gram of malt in one hour at 30° C.

Application of the beta-amylase method to barley malts is discussed. The lack of parallelism between alpha- and beta-amylase contents is demonstrated as well as the variation in the fraction of malt saccharogenic activity attributable to beta-amylase.

Literature Cited

Blish, M. J., and Sandstedt, R. M.
1933 An improved method for the estimation of flour diastatic value. Cereal Chem. **10**: 189–202.

Blom, J., Bak, A., and Braae, B.
1937 Alpha-amylasen—beta-amylasen. Z. physiol. Chem. **250**: 104–122.

Freeman, G. G., and Hopkins, R. H.
1936 The mechanism of degradation of starch by amylases. II. Kinetics of action of alpha- and beta-amylases on starch, its components and partial degradation products. Biochem. J. **30**: 446–450.

Hills, C. H., and Bailey, C. H.
1938 The amylases of different barley varieties. Cereal Chem. **15**: 351–362.
Ohlsson, Erik
1926 On the two components of malt diastase. Compt. rend. trav. lab. Carlsberg **16** (No. 7): 1–68.
1930 Über die beiden Komponenten der Malzdiastase, besonders mit Rücksicht auf die Mutarotation der bei der Hydrolyse der Stärke gebildeten Produkte. Z. physiol. Chem. **189**: 17–63.
—— and Uddenberg, C. E.
1933 Amylasen in ruhenden und keimenden Samen. II. Roggen. Z. physiol. Chem. **221**: 165–173.
Sallans, H. R., and Anderson, J. A.
1938 Varietal differences in barleys and malts. II. Saccharifying activities of barleys and malts and the correlations between them. Can. J. Research **C16**: 405–416.
Sandstedt, R. M.
1937 The adaptation of the ferricyanide maltose method to high diastatic flours. Cereal Chem. **14**: 603–604.
—— Kneen, Eric, and Blish, M. J.
1939 A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16**: 712–723.
Stenstam, T., Björling, C. O., and Ohlsson, Erik
1934 Amylasen in ruhenden und keimenden Samen. IV. Weizen. Z. physiol. Chem. **226**: 265–271.

REPORT OF THE 1939–40 COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

HOWARD M. SIMMONS, *Chairman*

The Mid-West Laboratories Co., Inc., Columbus, Ohio

(Read at the Annual Meeting, May 1940)

The program followed by the 1939–40 Biscuit and Cracker Committee was planned to deal with the three recommendations of the previous committee (Simmons, 1940): (1) That further work of this same type be done to build up a greater volume of data to correlate more closely the laboratory evaluation of cracker flours with their actual shop performance. (2) That further study be made on the scoring of crackers in order to obtain a closer agreement among collaborators. (3) That a statistical study be made of all pertinent data accumulated by the committees in the last three years, this to be done in order that more practical conclusions may be reached.

The statistical study is being prepared and will be given in a separate report. Work done by the last four committees indicates that "The quality of the crackers seemed to depend upon the type of flour used in the sponge rather than upon the dough flour used in the formulae" (Reiman, 1937; Brown, 1938; Simmons, 1939). With this in mind, the committee selected three types of sponge flour and one dough flour.

The chemical analysis of each flour was made by two members of the committee, while the hydrogen-ion concentration was made by one

member. Viscosity tests were made by each member of the committee, following the methods outlined by Bayfield (1936), procedures No. 1 and No. 2. On the basis of his own viscosity tests and the analytical data furnished with the samples, each committee member classified the samples. Bread was baked by one member of the committee, using the regular A. A. C. C. method and a loaf from each flour was sent to each committee member for classification as to use as a sponge and/or dough flour.

Crackers were baked in a commercial shop, following schemes used by Reiman (1938) and Brown (1939); *i.e.*, the three sponge flours were baked with the dough flour and in addition the weak sponge flour was used as a dough flour. Three cracker bakes were made at three-week intervals. Each member of the committee scored and evaluated the crackers according to the method outlined in *Cereal Chemistry* **15**: 37. One member of the committee analyzed the baked crackers and another determined the shortometer values.

For the sake of continuity, the flours were designated as Nos. 17, 18, 19, and 20. Table I gives the analytical data and the type of wheat from which each flour was milled.

TABLE I

ANALYTICAL DATA AND FLOUR TYPE

Sample No.	Protein (15% H_2O)	Ash (15% H_2O)	pH	Maltose [1]	Used for	Wheat used
	%	%		*mg.*		
17	9.19	.406	5.75	157	Strong sponge	Illinois Red
18	8.56	.408	5.83	125	Sponge	Indiana Red
19	8.80	.392	5.81	134	Sponge	Ohio Red
20	7.76	.395	5.80	116	Dough	Indiana Red

[1] Maltose determination made by W. H. Hanson.

Discussion of Results

This year's results showed exceptionally close agreement by the collaborators on their viscosity results, both by the no-time method and the one-hour digestion method. In only one case was the variation greater than ± 3 degrees from the average. Since this committee is national, it speaks well for the progress which has been made in the viscosity test, both by the one-hour digestion and the no-time method. Table II gives the average by both methods.

It should be noted that, although milled from wheat grown in two different states, flours No. 18 and No. 19 showed almost identical viscosities, both by the one-hour and the no-time method.

Table III gives the average individual classification of flours based on the viscosity tests. The classification was made from the final read-

ings respectively, from both the one-hour and the no-time method. The agreement between collaborators was so close that only the average is shown.

TABLE II

AVERAGE VISCOSITY DETERMINED BY THE ONE-HOUR AND THE NO-TIME METHOD

	Sample number and method							
	17		18		19		20	
	1-hour	No-time	1-hour	No-time	1-hour	No-time	1-hour	No-time
Average	116.6	86.4	92	71.7	92	70	61.8	48.5

TABLE III

AVERAGE CLASSIFICATION OF FLOURS ON BASIS OF ANALYTICAL AND VISCOSITY TESTS

	Sample number			
Method	17	18	19	20
1-hour	Strong to very strong sponge	Sponge to strong dough	Sponge to strong dough	Dough
No-time	Strong sponge	Sponge to strong dough	Weak sponge to strong dough	Dough

In two cases the flours were rated one degree higher by the one-hour method than by the no-time method. This confirms last year's report (Simmons, 1939): "The one-hour method places the border-line flours slightly higher than the no-time method."

The baking test classification (Table IV) follows very closely the classification of these flours by the viscosity tests. The classification probably parallels the viscosity results of the one-hour method more closely than the no-time method. There was not the tendency, this

TABLE IV

CLASSIFICATION OF FLOURS BY THE BAKING TEST

	Sample number			
Collaborator	17	18	19	20
No. 1	Strong sponge	Sponge	Sponge	Strong dough
No. 2	Very strong sponge	Sponge	Sponge	Strong dough
No. 3	Strong sponge	Sponge	Sponge	Dough
No. 4	Sponge	Weak sponge	Weak sponge	Weak dough
No. 5	Strong sponge	Sponge	Sponge or dough	Dough
No. 6	Sponge	Sponge or dough	Sponge or dough	Dough
No. 7	Sponge	Sponge or dough	Sponge or dough	Dough
No. 8	Strong sponge	Medium sponge	Weak sponge or dough	Dough

year, to place the borderline flours a degree higher by the baking test, as was noted in the preceding reports. This may be due to the fact that all of these flours showed low diastatic values. Since this factor was not determined by preceding committees, no conclusions can be drawn. It might be well in future work of this type to incorporate this determination.

Flour No. 19, which showed almost the same viscosity by both methods as No. 18, was rated by the majority as a weak sponge or dough flour by the baking test, while No. 18 was rated as a sponge flour.

The same tendency was noted this year, as to the individual scoring of crackers, that "There is a considerable difference between the way individuals score crackers, both in valuation and their rating."

Table V shows the ranking of the crackers baked from the four

TABLE V

RANKING OF CRACKERS BY SCORES AND SHORTOMETER VALUES

Collaborator	First bake Samples A	B	C	D	Second bake Samples 2	1	4	3	Third bake Samples IV	III	II	I
No. 1	3	2	1	4	1	3	2	4	4	1	3	1
No. 2	4	3	1	2	1	4	2	3	4	3	1	2
No. 3	3	2	4	1	3	4	1	2	3	2	1	4
No. 4	4	1	1	2	2	2	1	2	2	3	1	4
No. 5	4	3	1	2	1	4	3	2	1	3	2	4
No. 6	2	1	3	4	4	2	1	3	3	4	2	1
No. 7	3	2	4	1	4	1	3	2	2	3	4	1
No. 8	4	2	1	3	4	3	1	2	3	2	1	4
Total	27	16	16	19	20	23	14	20	22	21	15	21
Rank	4	1	1	2	2	4	1	2	4	2	1	2

	SUMMARY: Samples A	B	C	D	SHORTOMETER RANK: Samples A	B	C	D
First bake	4	1	1	2	2	3	1	4
Second bake	2	4	1	2	2	3	1	4
Third bake	4	2	1	2	3	1	2	4
Total	10	7	3	6	7	7	4	12
Rank	4	3	1	2	2	2	1	4

flours. Although there is a difference between individuals in evaluation and rating, the total ranking of crackers this year shows close agreement for the three bakes.

The *A* cracker, which ranked fourth on two of the bakes, was baked with the No. 17 flour used as a sponge. This flour was rated by both the baking test and the viscosity by the one-hour digestion method as a "strong sponge" to "very strong sponge" flour. This would confirm the observation made last year (Simmons, 1939), that "Viscosity by

the one hour digestion method more properly classifies this flour than the no time method."

The *C* cracker, which ranked first on all three bakes, was baked with the No. 19 flour as a sponge flour. That flour was rated as a slightly weaker flour by the baking test than the No. 18 flour which was used as a sponge flour in baking the *B* cracker. The *B* cracker ranked third, although placed equal to *C* on the first bake.

The No. 19 flour was used as both a sponge and a dough flour in the *D* cracker, and although classed as a sponge or dough flour by the baking test, the ranking of the crackers indicates that it offered fewer problems as a sponge flour.

The results of the cracker bakes tend to further confirm the observations made by Reiman (1937) and Brown (1938), that "The quality of crackers seems to be more dependent upon the type of sponge flour than the type of dough flour used in the formula." Even though the *D* cracker was baked with a very strong dough flour it rated second.

The shortometer results are not so gratifying as they might have been, except on the *B* and *C* crackers, where they agreed with the ranking of crackers by the collaborators. The *D* cracker rated fourth on all three bakes by the shortometer, while the *A* cracker ranked fourth by the collaborators and second by the shortometer.

The chemical and physical analyses of the crackers (Table VI), while

TABLE VI

CHEMICAL AND PHYSICAL ANALYSES OF CRACKERS[1]

Bake No.	Cracker	Moisture	Protein (N×6.25)	Ash	Fat	Shortometer value	Av. pH	Av. thickness	Av. count per lb.
		%	%	%	%			*In.*	
1	A	5.45	9.37	2.21	13.14	51	8.5	3	127
	B	5.85	8.84	2.59	12.93	53	8.55	2 15/16	117
	C	4.95	9.19	2.71	13.11	48	8.6	2 14/16	123
	D	5.90	9.63	2.47	13.13	59	8.6	2 14/16	118
2	A	5.80	9.70	2.22	13.19	59	8.4	3 2/16	107
	B	7.70	9.30	1.79	12.08	64	7.8	2 14/16	114
	C	7.00	9.00	2.36	13.14	55	7.8	2 15/16	118
	D	5.20	9.00	2.49	13.06	65	8.3	3	109
3	A					49	8.6	3 3/16	114
	B					43	8.4	2 15/16	123
	C					48	8.3	2 15/16	123
	D					61	8.3	3 1/16	115

[1] Chemical analyses by T. E. Hollingshead; shortometer values by H. J. Loving.

not differing greatly from those reported by previous committees, did show some points of variation. The average pH of the cracker seemed to be somewhat higher, as was also the count per pound. There was a

marked difference in the shortometer values which were expressed in 32nds of a pound in all cases. This year the values are between 49 and 65, while last year the values were between 63 and 90, and two years ago Brown's (1938) report showed values between 56 and 80.

Last year the committee observed that "The higher the moisture content the higher the shortometer value." This seemed to hold true in most cases this year.

Conclusions

Although the work done by the committee this year has been essentially a repetition of the work done by previous committees, it is felt that the results have served as a substantial confirmation of the work of these committees on evaluation of flours by the baking and viscosity tests.

A closer agreement has been secured between collaborators on the viscosity tests and on the scoring of baked crackers.

Recommendations

The committee recommends that further work on cracker flours be suspended until the statistical study can be brought up to date. This report is to cover the five years' work including the work of the committee this year.

It is also recommended that work be done on biscuit or cookie type flours in order to bring the testing of this class of flours to the present status of the cracker flours.

Acknowledgments

The chairman wishes to express his thanks to the Kroger Grocery and Baking Company for their cooperation and to W. S. Culver, who cooperated in securing the flours and supervising the craker bakes.

To the members of this committee, C. C. Armuth, W. H. Hanson, T. E. Hollingshead, H. J. Loving, H. O. Triebold, O. P. Skaer, and Miss Pearl Brown, the chairman also wishes to express his appreciation and thanks.

Literature Cited

Bayfield, E. G.
1936 Report of subcommittee on viscosity. Cereal Chem. **13**: 773–788.

Brown, Pearl
1938 1937–1938 report of subcommittee on testing biscuit and cracker flours. Cereal Chem. **16**: 130–136.

Reiman, W.
1937 1936–1937 report of subcommittee on testing biscuit and cracker flours. Cereal Chem. **15**: 35–48.

Simmons, H. M.
1939 1938–1939 report of subcommittee on testing biscuit and cracker flours. Cereal Chem. **17**: 250–257.

REPORT OF THE 1939–40 COMMITTEE ON METHODS OF TESTING SELF-RISING AND PHOSPHATED FLOURS

O. E. GOOKINS, *Chairman*

The Quaker Oats Co., St. Joseph, Missouri

(Read at the Annual Meeting, May 1940)

Committees of former years have provided a formula, methods of baking, a scorecard, and definitions and evaluations of score-card terms. A series of photographs representing a range of textures and also a method of preparing semipermanent color standards for biscuit scoring have been presented. Studies on variations due to ovens and effects of varied absorptions have been made. Numerous collaborative bakings, with different types and kinds of flours, have shown considerable variability in scoring. The use and purpose of biscuit-baking tests have been expounded, and attempts to arrive at specifications for biscuit-flour standards have been reported.

By collaborative baking and by questionnaires, this committee endeavored to determine two things: (1) whether baking to a uniform oven loss would be a means of bringing collaborators to a common basis that would result in more uniform biscuit volumes, and (2) specifications for standard flours to be used to smooth out personal, machine, and atmospheric differences in performing the biscuit baking test.

Samples of three self-rising flours, with entirely different characteristics, were sent to each of the collaborators. Sample No. 1 was a hard-wheat patent, 10.5% protein; sample No. 2 a soft-wheat patent, 9% protein; sample No. 3 a soft-wheat cake flour, 7.5% protein. The pH of No. 1 was 5.5; No. 2, 5.4; while No. 3, which was chlorine bleached, had a pH of 4.9. Viscosities of these flours were: No. 1, 229°; No. 2, 98°; No. 3, 27° MacMichael. Ash contents, respectively, of these flours were: No. 1, 0.38%; No. 2, 0.34%; No. 3, 0.32%.

Self-rising ingredients were mixed with these flours, using the formula given in *Cereal Chemistry* by Walter (**13**: 722): flour 100, hydrated monocalcium phosphate 1.875, sodium bicarbonate 1.5, salt 2.0

Specific directions for baking and scoring the biscuits were given. Each collaborator was asked to maintain a 14% oven loss, which was 1% above the average oven losses in the baking tests of the 1938–39 committee. Sufficient flour for three bakes was sent to each collaborator.

Results of the baking tests which were reported by three of the collaborators, as given in Tables I, II, and III, indicated rather definitely the need for a standard flour to be used as a basis of comparison

in order to obtain closer agreement on items of the score card. While the actual scores varied considerably, the relative ranking of the three flours for the various score-card items showed some semblance of agreement. In the tenderness ranking the three reporting collaborators agreed that flour No. 3 was the best. Flavor scores varied consider-

TABLE I

SELF-RISING SAMPLE NO. 1 (HARD PATENT)

Standard score		Collaborators A	B	C	Average score
Grain	10	9.8	9.0 heavy cell	7.0	8.6
Tenderness	10	8.8	8.5	8.5	8.6
Flavor	20	19.8	17.0	17.5	18.1
Crumb color	20	17.0	16.4 creamy	15.0	16.1
Volume [1]	40	41.8	41.6	41.4	41.6
Total	100	96.2	92.5	89.4	93.0
Water used		162 cc.	160 cc.	156 cc.	159 cc.
pH biscuit		Col. 7.15 El. 7.15		Col. 7.0 El. 7.3	7.15
Oven loss [2]		13.9	14.6	15.3	14.6
Spec. volume [3]		2.09	2.08	2.07	2.08
No. bakes		3	3	2	—

[1] $\frac{\text{cc. biscuit}}{\text{g. dough}} \times \frac{40}{2}$.

[2] $\frac{\text{wt. dough} - \text{wt. biscuit}}{\text{wt. dough}}$.

[3] $\frac{\text{cc. biscuit}}{\text{g. dough}}$.

TABLE II

SELF-RISING SAMPLE NO. 2 (SOFT PATENT)

Standard score		Collaborators A	B	C	Average score
Grain	10	10	9.5	8.5	9.3
Tenderness	10	10	9.0	7.0	8.7
Flavor	20	20	19.0	17.5	18.8
Crumb color	20	20	18.3	17.0	18.4
Volume	40	42.2	40.6	40.2	41.0
Total	100	102.2	96.4	90.2	96.2
Water used		156 cc.	148 cc.	154 cc.	153 cc.
pH biscuit		Col. 7.15 El. 7.15		Col. 7.0 El. 7.33	7.15
Oven loss		14.0	14.4	13.63	14.0
Spec. volume		2.11	2.03	2.01	2.05
No. bakes		3	3	2	—

TABLE III

SELF-RISING SAMPLE NO. 3 (SOFT CAKE-FLOUR PATENT)

Standard score		Collaborators A	B	C	Average score
Grain	10	10	8.4 weak, close	9.0	9.1
Tenderness	10	12	10.0 short	9.0	10.3
Flavor	20	19.2	18.5 dry	18.0	18.6
Crumb color	20	22	19.0 dead	18.0	19.7
Volume	40	39	37.6	43.2	39.9
Total	100	102.2	93.5	97.2	97.6
Water used		149 cc.	136 cc.	152 cc.	146 cc.
pH biscuit		Col. 7.05	El. 7.03	Col. 7.0 El. 7.26	7.07
Oven loss		15.4	15.2	14.88	15.2
Spec. volume		1.95	1.88	2.16	1.96
No. bakes		3	3	2	—

ably; individual tastes and perhaps the differences in time that elapsed before baking by the different collaborators may have affected these. Color-of-crumb scores ranked the flours in the same order in each case, with flour No. 3 first, No. 2 second, and No. 1 third. Volume scores agreed very well on Nos. 1 and 2. Sample No. 1 ranged from 41.4 to 41.8 and No. 2 from 40.2 to 42.2. On No. 3 the collaborators did not check so well, their scores ranging from 37.6 to 43.2.

The pH of the biscuits checked well, averaging 7.15, 7.15, and 7.07 for Nos. 1, 2, and 3, respectively.

Oven losses were maintained reasonably close to 14%, with a maximum variation of $\pm 1.4\%$ for all collaborators on the three samples. There is probably insufficient collaborative data here to say definitely whether controlled oven loss points toward any better agreement on volume. Samples No. 1 and No. 2 tend to support this supposition, while No. 3 would disprove it. Additional tests in the Quaker Oats laboratory gave similar specific volumes over a range of 5% oven loss. The tendency of the 1938–39 committee's collaborative bakings to show lower specific volumes with lower oven losses was not borne out in the above-mentioned tests.

It is our belief that the kind of mixing and baking equipment used, the technique and experience of the operator, and the volume-measuring device are the three most important items affecting volume values. The time and type of storage of the self-rising flour prior to baking are also factors which affect volume. It is our opinion that controlled oven loss is *not* the answer for better agreement in volume scoring.

The second item in this committee's problem for the year was the determination of specifications for standard flours. Opinions on this were obtained by questionnaires and by reference to previous committees' ideas. The need for a standard or standards has been known for several years. Most suggestions agreed that a short-patent bleached soft-wheat flour of approximately 9% protein, 5.3 to 5.6 pH, and under 0.36 ash, should constitute one standard. The use of supplementary standards, such as a soft straight and a soft clear, and also a hard-wheat short-patent, were suggested. It is the general opinion that the soft-wheat short-patent flour should be one for whose grade the standard score totals 100. The flour should have a good color.

The question was raised by a member of the committee as to whether the recommendation of self-rising flour standards falls within the province of this committee. The problem is complicated by the wide variety of self-rising and phosphated flours on the market today. The writer concurs in this point, that it is impractical to try to set up a definite standard or standards to fit everyone's need. It is suggested that the few specifications given for a soft-wheat patent flour standard be adopted as a guide only, and that in any collaborative baking tests a flour of such type be sent along and specified as the standard to be used in judging the other samples.

In conclusion, this year's committee has, by questionnaire and by collaborative baking tests, first, found that controlled oven loss does not seem to result in more uniform biscuit volumes; second, suggested a few specifications on a soft-wheat short-patent flour to be used as a guide in selecting a standard flour for scoring biscuits, and, further, suggested that in all collaborative biscuit baking a flour of such type be sent and designated as standard.

Acknowledgments

The chairman wishes to acknowledge gratefully the cooperation of the committee: R. A. Barackman, L. G. Brown (deceased), Elizabeth McKim, G. W. Pearcy, F. R. Schwain, and C. C. Walker.

REPORT OF THE 1939-40 COMMITTEE ON METHODS OF TESTING CAKE FLOUR

J. W. MONTZHEIMER, *Chairman*

Centennial Flouring Mills Co., Spokane, Washington

(Read at the Annual Meeting, May 1940)

Our program for the past two years has been directed towards a comparison of different cake test formulas and the behavior of various cake flours.

Table I gives percentages of ingredients in the various formulas

TABLE I

PERCENTAGES OF INGREDIENTS USED IN FORMULAS BY 1938–39 CAKE COMMITTEE

Formula	Flour	Sugar	Shortening	Eggs	Milk	Salt	Baking powder
A.A.C.C.	28.89	27.78	7.22	9.11 [1]	25.56	0.44	1.0 [2]
Modified	28.86	27.74	7.22	9.12 [1]	25.51	0.44	1.11
2	27.7	27.7	11.0	9.9 [1]	22.0	0.6	1.1
5	26.52	26.52	15.15	17.05 [3]	13.45	0.75	0.56
6	26.14	26.14	13.07	13.07 [3]	19.60	0.8	1.18 [2]
1	25.7	30.6	9.4 [4]	14.00 [1]	18.0	0.9	1.4
4	24.62	29.55	9.4	14.77 [1]	19.70	0.78	1.18
10	23.92	28.70	9.56 [4]	14.36 [1]	21.54	0.77	1.15
3	21.09	30.00	11.72 [4]	12.65 [3]	22.48	0.75	1.31
7	21.06	29.49	11.50 [4]	15.84 [1]	20.25	0.81	1.05

[1] Egg whites.
[2] Used soda and cream of tartar.
[3] Whole eggs.
[4] Special cake shortening.

used. The formulas are grouped according to the percentage of flour contained in the batter, which ranges from 28.89% in our present A.A.C.C. test formula down to 21.06% in the formula at the bottom of the list, which is made with a special cake shortening. It is worthy of note that the percentage of sugar in the batters varies from 26.14% to 30% and that the so-called "hi-ratio" cakes contain only 4% more sugar in the batter than the lean-formula cakes. The really important change in the batter is the reduction of the flour from 5% to 7% when using the special type of shortening.

Special consideration was given by this year's committee to a new scoring method copied after the system being used in the canning industry. This is illustrated in Table II. The outstanding points in this system are:

1. Volume is not included in the score but measured in cubic centimeters.

TABLE II

PROPOSED SCORING SYSTEM

		Score			
		Excellent	Good	Fair	Poor
Symmetry	15	15–13	12–10	9–6	5 or less
Crust	5	5	4	3	2 or less
Texture					
Tenderness	15	15–13	12–10	9–6	5 or less
Silkiness	15	15–13	12–10	9–6	5 or less
Grain	25	25–22	21–18	17–14	13 or less
Color	15	15–13	12–10	9–6	5 or less
Flavor (taste and eating quality)	10	10–9	8–7	6–5	4 or less

2. Consideration is given to flavor as part of the score.

3. A so-called "stop" system is used. In the "stop" system each unit of the score is divided into four classifications: excellent, good, fair, and poor. A certain percentage of the score is allotted to each classification. A cake to grade excellent would necessarily have to grade excellent for every unit of the score. In our present system this would not be necessary. For instance: should a cake grade excellent in all but color and the color grade below five, which would be considered poor, the final score on the cake would be noted as poor on account of color. The same rule would apply to any of the characteristics of the cake, such as symmetry, texture, or grain.

Table III gives the analysis and the description of the flours used in this year's work.

TABLE III

ANALYSIS ON FLOURS[1]

1939 CAKE COMMITTEE FLOURS

A—Commercial cake flour.
B—Commercial cake flour.
C—Commercial pastry flour, chlorine bleach.
D—Commercial all-purpose family flour, Agene bleach.

Sample	Protein	Ash	Moisture	pH 30°	Viscosity		
					20 g. flour	2 g. protein	No time
	%	%	%		°*MacM.*	°*MacM.*	°*MacM.*
"A"	7.5	.348	11.9	5.24	54	108	41
"B"	7.4	.368	12.0	5.08	47	111	34
"C"	7.4	.415	11.7	5.40	44	97	29
"D"	10.3	.402	12.3	5.80	136	124	109

[1] Prepared by V. H. Morris, Ohio Agricultural Experiment Station.

Cakes were baked using the present A.A.C.C. formula and a modified A.A.C.C. formula set up with 90 cc. of egg white and 90 g. of

shortening. Both layer and loaf cakes were baked from each batter. Cakes were judged according to the present A.A.C.C. score system and the proposed score system (Table II), use being made of Stamberg's photographs for grain and symmetry judging in this work.[1]

Character of the flours was varied over a large enough range so that grain and texture differed considerably. Using either loaf or layer cakes and reporting by either scoring system, all members of the committee classified the cakes in about the same order, there being some difficulty in deciding on flours A and B between collaborators.

Summary

Committee members disagreed on the value of layer and loaf cakes for testing.

No agreement was reached as to whether volume should be reported in the score or merely by number of cubic centimeters.

Few members favor the use of flavor as part of the score card.

Members all agreed on value of "stop" system in scoring.

Recommendations for Future Committees

Immediate steps should be taken to provide descriptive terms for present score card.

Further study should be made on a "stop" system for the present score card.

Both layer and loaf cakes should be considered.

Measurement should be made on pH of the finished cake.

In scoring grain: (a) an attempt should be made to further complete the Stamberg photographs, and (b) cake slices should be prepared for scoring.

The break on top of a loaf cake should be considered when scoring symmetry.

Further attempts should be made to standardize on equipment between collaborators.

Acknowledgments

Work this year was carried on by the following members of the Cake Committee: W. E. Stokes, R. W. Mitchell, F. J. Coughlin, William Haley, Lowell Armstrong, and O. E. Stamberg.

The committee wishes to acknowledge the assistance of Donald Wade of Procter & Gamble Co., Mr. King of Fisher Flouring Mills Co., and Mr. Jolitz and Mr. Putnam of General Foods Corporation.

[1] Cereal Chemistry 16: 764.

REPORT OF THE 1939–40 COMMITTEE ON THE STANDARDIZATION OF LABORATORY BAKING

QUICK LANDIS, *Chairman*

The Fleischmann Laboratories, 810 Grand Concourse, New York, N. Y.

(Read at the Annual Meeting, May 1940)

The impending revision of the Book of Methods has forced the committee to a somewhat hurried consideration of the whole aspect of the baking test. Steady progress has been made since the last revision and many of the improved features are sufficiently well established to permit their incorporation into the method.

In view of the difficulties which have arisen with respect to the availability of a single mixer, particular emphasis has been placed on calibration. To facilitate calibration it would be desirable to have an "analyzed" sample of flour continuously available to our members. The Association might wish to consider this possibility.

Satisfactory methods for reporting and interpreting results are not yet well developed. Characterization of loaf types and internal characteristics as an aid to this problem should be included in the Book of Methods. The committee will undertake this project and hopes to have it ready in time to incorporate in the revision.

Although the pan situation is still unsatisfactory the committee has as yet insufficiently complete data to warrant recommendation of change in the pan sizes previously specified.

The committee has concluded that the rolling pin method of hand manipulation is definitely superior to that previously described, and thus recommends the substitution of the improved method when machine method molding is not available.

Accordingly it is recommended:

1. That Cereal Laboratory Methods be amended according to the recommendations submitted.

2. That the Association consider the appointment and maintenance of a committee to prepare, analyze, and periodically refresh a sample of flour to serve for mixer calibration.

3. That the succeeding baking test committee continue its efforts toward the development of the baking test with particular emphasis on reporting of results.

BOOK REVIEW

Official and Tentative Methods of Analysis. Association of Official Agricultural Chemists, Washington, D. C., 1940. 757 pages.

This new edition continues the tradition of the Association of Official Agricultural Chemists of selecting the most reliable methods of chemical and physical analysis used in regulatory and research work.

The book shows a marked expansion in the work of the Association. In the Fourth Edition, six chapters were given without text. The chapters on Fish and Other Marine Products, Vitamins, Microbiological Methods, and Microchemical Methods have been developed in this edition, leaving only the chapters on Sewage and Agricultural Dust without text.

The increased activity of the Association has also resulted in numerous additions to the individual chapters. For example, the chapter on Cereal Foods has increased from 79 subdivisions in the Fourth Edition to 99 in the Fifth Edition. Methods for sugars (reducing and nonreducing) have been added to this chapter.

A different method for the determination of chlorine in fat is given. The increased use of soya beans has developed a need for the detection of soya flour, and a qualitative test is given for soya flour in uncooked cereal products. Similarly, the chapter on Grain and Stock Feeds has increased from 56 to 68 subdivisions. A method for determining fat acidity in grain has been added. A method is presented for the determination of manganese, the element needed in the diet of poultry to prevent perosis, or slipped tendon. The increased interest in vitamin A activity has resulted in the addition of a method for the determination of carotene in hay. After the extraction and separation of the carotene, three choices for estimation are given. Either a spectrophotometer, photoelectric colorimeter, or visual colorimeter can be used. A table is given for using potassium chromate solutions for standards of comparison for the latter two types of measurements.

Additional methods of analysis in the chapter on Dairy Products has resulted in an increase in the number of subdivisions from 110 to 135. The methods of analysis that have been added to this chapter include: the determination of citric acid in dried milk, a test for mold mycelia in butter, a method for gums in soft curd cheese, a phosphatase test for pasteurization, and bio-assay for vitamin D in milk.

The reviewer would not undertake to report all the changes in the three chapters cited. The examples were chosen merely to show the numerous additions and changes that the Association has made in this new edition. Similar additions and changes can be noted throughout the book.

The revision of subject material has necessitated an increase over the Fourth Edition of only 47 pages. This small increase in the size of the book has to some extent been made possible by the use of the term *ca* for *approximately* and *about*, the partial elimination of the articles *a*, *an* and *the*, and other methods of presentation employed by the editors to save space without the loss of clarity.

The book is worthy of replacing its predecessor on the bench of the chemist who is called upon to make analyses of farm products and related materials.

KENNETH T. WILLIAMS